TAMPERE UNIVERSITY OF TECHNOLOGY

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DEVELOPMENT OF A MICROFLUIDIC LIQUID HANDLING SYSTEM FOR WASTE WATER SAMPLE ANALYSIS

Master of Science Thesis

Examiners:
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Yleisesti lähidiagnostiikassa on tavoitteena pienentää näyte- ja reagenssimääriä. Pienempien tilavuuksien ja dimensioiden ansiosta myös analyysiajat saadaan lyhentymään. Mittausprotokollan automatisoinnilla saadaan vähennettyä diagnostiikkalaitteen käyttäjän osuutta. Myös kokonaiskustannukset pienenevät. Nämä voidaan toteuttaa esimerkiksi mikrofluidistiikan avulla, joka on keskeisimpiä miniatyrisointiteknologioita. Tällöin tutkitaan ja kehitetään pieniä nestemääriä käsitteleviä laitteita, joissa ainakin yksi dimensio on mikrometreissä. Biomolekyylien detektoinnissa käytetään herkkiä lantanidi-ioneita merkkiaineina. Näistä erityisesti europium on yleisesti käytetty.

Tutkimuksen tavoitteena oli selvittää mikrofluidistisen polystyreenikasetin käyttömahdollisuuksista jätevesianalyysissa. Tavoitteena oli selvittää Eu-leimattujen polymeerien käyttäytymistä kanavarakenteissa ja detektointia staattisesta ja virtaavasta näytteestä. Lisäksi selvitettiin voidaanko samaa kasettia käyttää useampaan kertaan, jos mittauskertojen välissä suoritetaan kasetin pesu.

Tutkimusmenetelmä perustuu polymeeriin kiinnitetyn europiumkelaatin fluoresenssin detektioon. Eu-leimatut polymeerit aiheuttavat korkeita signaalitasoja ja analyysin herkkyys on hyvä. Detektointiin käytettiin aikaerotteisen fluoresenssin mittaukseen tarkoitettuja laitteita; Victor^2 monilevylukijaa ja projektin käyttöön muokattua laitetta (Hidex).

Tulosten perusteella voidaan todeta, että käytetty kanavarakenne toimii mitattaessa staattisesti ja virtauksen kanssa. Työssä todettiin, että mitattavan polymeerin adsorptiota kasettirakenteeseen voidaan välttää, jos mittausten välissä kasetti pestään dialyysipuskurilla. Signaalitasoja mitattiin myös virtauksen kanssa, jolloin saatiin selville, että mittauksen voi suorittaa virtaavasta näytteestä. Virtausmittausta varten muokattuun Hidexin mittalaitteeseen lisättiin letkutus ja näytteenotto kehitettiin venttiilien avulla sellaiseksi, että se voisi vastata todellista prosessista tapahtuvaa mittaustapahtumaa. Kaiken kaikkiaan voidaan todeta, että polymeerin määrää voidaan mitata virtaavasta näytteestä samaa mittakasettia käyttäen, kunhan mittauskammio pestään mittausten välissä.

ABSTRACT

TAMPERE UNIVERSITY OF TECHNOLOGY
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In the point-of-care diagnostics, one of the trends is goal to decrease the amounts of samples and reagents. Also the times which are used to analysis are decreased because of smaller volumes and dimensions. Automation of the measurement protocol is used to decrease the involment of the user when diagnostic equipment is used. Also the costs are decreasing. All this can be carried out with microfluidics which is one of the most important areas of miniaturization technologies. In microfluidics, equipment with small fluid volumes are studied and developed. A microfluidic device has at least one dimension which is measured in micrometres. Sensitive lanthanide ions are typically used as markers when biomolecules are detected. Especially europium is commonly used.

The objective of this research is to study the use of microfluidic polystyrene cartridge in waste water analysis. More specific goals are to study how Eu-labeled polymer particles behave in a microfluidic channel and detection from static and flowing sample. The cartridge reusability when the cartridge is washed between the measurements is also studied.

The research protocol used is based on detection of fluoresence of an Eu-chelate which is attached to the polymer. The Eu-labeled polymers produce high signal levels and provide good sensitivity. Two time-resolved fluorescence measurement equipment are used for detections; Victor^2 multiplate reader and an equipment modified for this study (Hidex).

Based on studies, the channel structure works well during the static and flow measurements. Polymer adsorption to the channel walls can be avoided if dialyse buffer is used for washing between the measurements. The results show that measurement is possible from a flowing sample. For the flow measurement is developed the sampling system which corresponds the measurement which could be performed from the real process. The conclusion of the thesis is that polymer remains can be measured from a flowing sample with a reusable cartridge, when the measurement chamber is washed between the measurements.

PREFACE

This thesis has been made as a part of a Tekes-funded NucleoTracker-project. The research has been carried out in Micro- and Nanosystems Research Group at the Department of Automation Science and Engineering at the Tampere University of Technology.

I wish to thank my examiner Prof. Pasi Kallio for the position as a research assistant in his group and for his advice in my work. I thank my examiner Prof. Ilpo Vattulainen for advice during the writing.

I thank my coworkers for their support, good ideas and pleasant conversations during the project. Especially I thank Jari for his advices in the practical work and Mathias for the user interface. The working atmosphere has been supportive and it has been fun to be a part of such a nice group.

I wish to thank my friends for all these great years. I thank my parents and siblings of their love and support for their engineer. Finally, I thank my fiancé of his love and patience for all these years and hopefully there are many more years left.

Tampere, May 17th, 2013

Hilla-Maria Keijälä

CONTENTS

ADDREVIATIONS

AFS	atomic fluorescence spectrometry
Av.	average
CSP	cylindrospermopsin
CV %	variation coefficient
DI water	deionized water
EDC	endocrine disrupting compounds
EDTA	ethylenediaminetetraacetic acid
Eu	Europium
HPMA	hydrolysed polymaleic anhydride
LOD	limit of detection
MCE	microchip electrophoresis
MEMS	microelectromechanical systems
MST	microsystem technology
µFIA	microflow injection analysis
NO_2	nitrogen dioxide
PAA	polyacrylic acid
PDMS	polydimethylsiloxane
PMMA	poly(methyl methacrylate)
PMT	photomultiplier tube
POC	point-of-care
PS	polystyrene
SD	standard deviation
SERS	surface-enhanced Raman spectroscopy
SPE	solid phase extraction
SO_2	sulfur dioxide
STX	saxitoxin family toxins
TNT	trinitrotoluene
TRF	time-resolved fluorescence
UV-light	ultraviolet light

SYMBOLS

A	cross-sectional area of channel
α	collision efficiency factor
β	collision frequency factor
C_{ij}	parameter between interacting molecules i and j
D	diffusion constant
D_H	hydraulic diameter
D_{ij}	parameter between interacting molecules i and j
d_i, d_j	collision diameters of aggregates belonging to sections i and j
ϵ	characteristic energy
η	fluid viscosity
f	friction coefficient
G	average velocity gradient or shear rate
γ	breakage distribution function
$\gamma_{lg}, \gamma_{sg}, \gamma_{sl}$	interfacial surface tension between liquid and gas, solid and gas, and solid and liquid
k_b	Boltzmann's constant
k_f	rate of spontaneus emission of radiation
k_i	rate of exited state decay
l	separating distance of molecules
μ	dynamic viscosity
$n(V,t)$	number of concentration of particles of aggregates
ν	kinematic viscosity
Δp	pressure difference
Φ	fluorescence quantum yield
Q	volumetric flow rate
r	radius
Re	Reynolds number
ρ	fluid density
S	specific rate constant
σ	characteristic length
T	temperature
t	time
θ	contact angle
V_{ij}	Lennard-Jones potential
V_1, V_2	particle or aggregate volumes
v	mean velocity
$< x^2 >$	average square displacement

1. INDRODUCTION

Microfluidic technology is a quite new branch of science and it is developing rapidly all the time. Microfluidics has been hot topic over 15 years and it produces numerous publications and patents every year. An explosion in biotechnology has also risen up microfluidics in automated analytical system. Microfluidics deals with fluids (gases and liquids) and handling micro volumes of these fluids. This kind of technology is suitable for biotechnology and for example water analysis, because analysis times are short, small volumes of sample are needed and analysis will be sensitive through automation. [1]

1.1 NucleoTracker project overview

This thesis is a part of a Tekes funded NucleoTracker-project. The project is started in the beginning of 2010 and will end in the spring of 2013. The project is co-operation with three universities: Tampere University of Technology (TUT), University of Turku (UTU), Åbo Akademi (ÅA), and five companies: Finnzymes Oy, Kemira, Plastone Oy, Hidex Oy and Thermo Fisher Scientific, Inc.

The main tasks (Fig. 1.1) is to develop rapid and simple-to-use real time technologies. More specifically, nucleic acid amplification test for water borne toxin pro-

Work Package 1: Nucleomics
O 1.1 Biological sample handling
O 1.2 Nucleic acid method
O 1.3 Polymer tracking method

Work Package 2: Reference methods development
O 2.1 Chromatographic reference methods for CSP and STX
O 2.2 Cyanobacterial culturing
O 2.3 Field sampling
O 2.4 Compliance with legislation

Work Package 3: Microfluidics
O 3.1 Automated sample handling method for O 1.1
T 3.1 Automated filtering method for O 1.1
T 3.2 Demonstration of filtering method with samples in laboratory
T 3.3 Development of portable instrument for O 1.2
T 3.4 Demonstration of filtering method with samples in field test
O 3.2 Development of microfluidic liquid handling for sample analysis
T 3.5 Basic material, flow and dissolution experiments with the process samples
T 3.6 Design and implementation of the microfluidic unit functions
T 3.7 Integration for the unit functions into a functional polymer tracking cartridge

Figure 1.1. *Work plan of the NuckleoTracker-project. This thesis has concentrated on the development of microfluidic liquid handling system.*

ducers that potentially produce *cylindrospermopsin* (CSP) and *saxitoxin family toxins* (STX), and a sensitive polymer tracking method for waste water analysis are studied.

This thesis covers development of microfluidic liquid handling for polymer tracking (Task 3.2). Task 3.2. is linked with Task 1.3. and it is separated into two main subtasks: basic material, flow and dissolution experiments with the process samples, and design and implementation of microfluidic unit functions needed in the polymer assays.

1.2 Overview of the thesis

The focus of the thesis is on the microfluidic assay cartridge and how the measurement is done. The objective of this thesis work is study how the europium labeled polymers behave in the microfluidic channels, how the measurement of europium intensity can be measured when the same cartridge is reused, and can the measurement be done with fluid flow. In the future this can be used as the quick test application for the waste water analysis.

Chapter 2 introduces the theoretical background including basics of microfluidics and the waste water process, and phenomena which are present in the measurement event. In Chapter 3, research methods, materials and equipment is introduced in more detail. Chapter 4 provides results with discussions, and Chapter 5 concludes the results of the thesis and introduces possibilities for the future research.

2. THEORETICAL BACKGROUND

In this chapter, the theoretical background of phenomena used in this thesis are introduced. First, there are the introduction of basic microfluidics phenomena and theories. Then across the phenomena of waste water process and water process in microfluidics is entered to polymers and an europium label and to fluorescence measurement. With these sections is created theoretical background for the measurement methods.

2.1 Microfluidics

Microfluidics is basically the flow of fluids in systems where at least one dimension is less than a millimeter. Area of microfluidics started at end of 1950s and development in the late 1980s dominated early stage of microfluidics. Microflow sensors, micropumps and microvalves are developed then. Microfluidics has become a hot topic and there are competing terms for it, such as "MEMS-fluidics", "Bio-MEMS" and "microfluidics" which describe the new research area with transport phenomena and fluid-based devices at microscopic length scales [2, p. 2]. Here the MEMS stands for the microelectromechanical systems, which are used today more for microtechnology, and microsystem technology (MST) is more for applications of fluidic and optical components. [2, pp. 1–2; 3]

Like said above, only the one dimension has to be in microscale and not all have to be shrinked to small scale. Actually only the area where fluid is processed has to be miniaturized. So the intrumentation size can be in other dimension. The key issue is the microscopic volume of fluid. With scaling laws phenomena are brought from macroscale to microscale. Can be seen that physics are same in both scales, but dominating effects are different. For example, in submicrometer scales the Brownian motion is the dominant transport phenomenon, but in macroscale it cannot be observed. [2, p. 2; 4]

Microfluidics has become the most dynamic segment of the MEMS because of its commercial potential. Practical applications from the microfluidic research are interest of the industry and the industry has taken part to the research. The microfluidic devices can be separated in fluid control devices, gas and fluid measurement devices, medical testing devices, like point-of-care (POC), and miscellaneous devices like implantable drug pumps. Major part of the microfluidic devices are

disposable applications. Companies can offer cheap devices which are available for everyone and huge market is secured. [2, pp. 4–5]

Usually the microfluidic applications are used in the places where users are not experts of the fluid physics. These kinds of applications are used by clinicians, cell biologists, police officers or public health officials. Different purpose of use sets different requirements for the applications and testing times can also vary when the purpose of use is changed. For different kind of environments of use, the applications have to be inexpensively available. One aim of the microfluidic applications is that they are disposable, quite cheap and easy to use. [4, 5]

2.1.1 Lennard-Jones -potential

The microfluidic system handles fluids, which can be either gas or liquid. The fluids have property of deformation and they can be easily deformed under external forces. Even low-magnitude shear forces can make large changes in the relative positions of the fluid elements. That is because liquids and gases have different densities, and the interactions between the molecules are freer than interactions in the solids. [2, pp. 11–12]

The interactions between the molecules can be described with the Lennard-Jones potential $V_{ij}(r)$ (Eq. 2.1) when two simple, nonionized and nonreacting molecules are the case.

$$V_{ij}(r) = 4\epsilon \left[C_{ij} \left(\frac{l}{\sigma} \right)^{-12} - D_{ij} \left(\frac{l}{\sigma} \right)^{-6} \right] \tag{2.1}$$

where l is the distance separating the molecules i and j, C_{ij} and D_{ij} are the parameters particular to their pair of the interacting molecules, ϵ is the characteristic energy scale and σ is the characteristic length scale. The term of l^{-12} describes the phenomenological model of pairwise repulsion. This repulsion is between two molecules when they are close enough. The term l^{-6} stands for the mildly attractive potential. This kind of weak interaction between the molecules is called van der Waals force. From Fig. 2.1 can be seen how intermolecular potential energy and force behave. The force between molecules is derived from Eq. 2.1. [2, p. 13]

In Fig. 2.1 can be seen how the magnitude of the potential and the force are decreased rapidly with distances beyond the location of the minimum. It is also seen that the curves never reach zero level. This point is very important when simulating molecular dynamics. [2, p. 14]

This Lennard-Jones interaction between the molecules is taken place in all three states of matter - solids, liquids and gases (Fig. 2.2). In the solids the molecules are in constant contact with certain distance σ between the molecules. This kind of crystal structure is densely packed. All molecules interact with their neighbor through

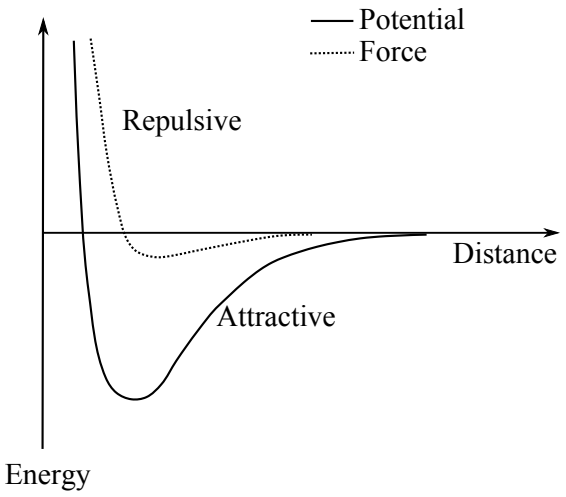


Figure 2.1. *Generalized plot of intermolecular potential energy and force according to the Lennard-Jones model. Adapted from [2, p. 14].*

the Lennard-Jones force. When the solid is heated to the liquid, the molecules are able to vibrate, but the distance between the molecules is still approximately σ. When the liquid is heated up, the vibration is increased, and after change of the state to the gas, the molecules jump energetically away from each other. Then the distance between the molecules is about 10σ at the standard conditions. [2, p. 15]

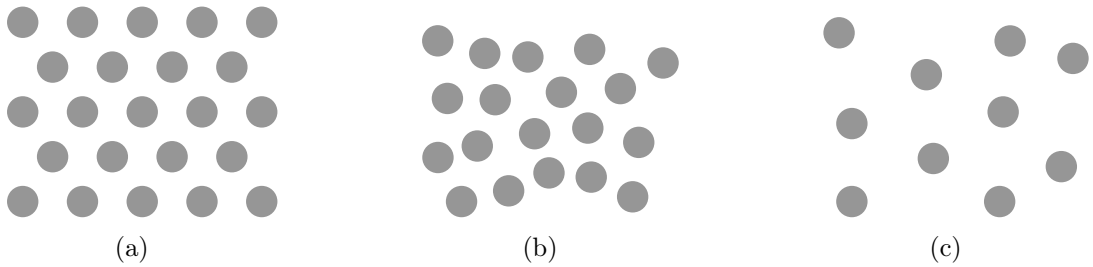


Figure 2.2. *Sketches of the three states of matter. The solid matter (a) has constant distance between molecules. In the liquid state (b) the molecules are able to vibrate, but the distance is still approximately σ. In the gas state (c) the distance between the molecules varies.*

In microfluidic point of view, the most interest is in the liquids and in the gases. In these two states of matter, the intermolecular forces are not so strong and they can be approached by classical physics. Generally in the fluid mechanics can be assumed that the fluid is treated as a continuum. There are enough molecules (thousands) even in microscales to consider the flow as continuum. [2, pp. 15–16]

2.1.2 Flow

Flow is motion of fluid and it can be laminar or turbulent. In the laminar flow the flow is smooth and the velocity is zero on the sides of the flux and the top velocity

is in the middle of the flux. In the turbulent flow the velocity varies. In Fig. 2.3 is shown these two cases, respectively.

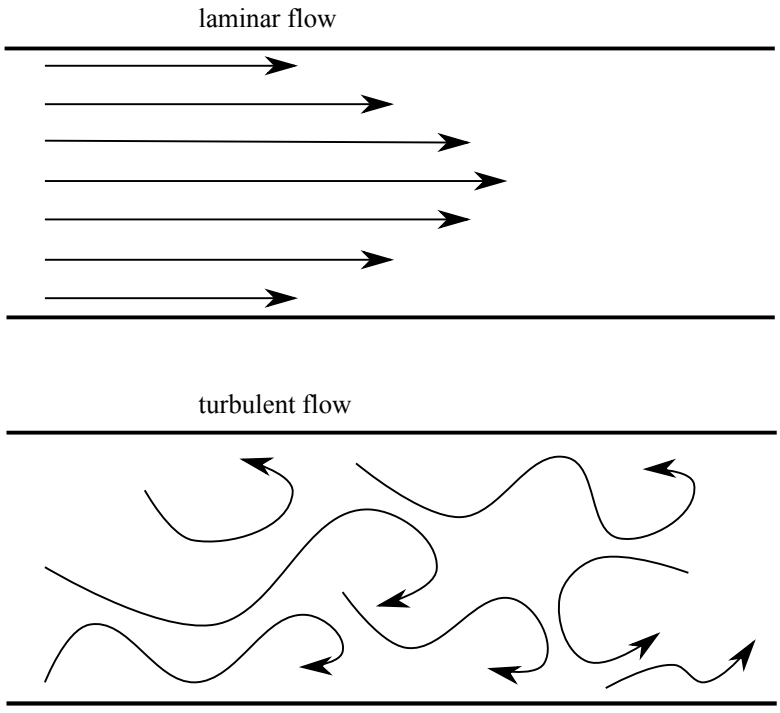


Figure 2.3. *The ideas of the laminar and the turbulent flow.*

In the microfluidic system the flow is laminar because of the dimensions. The flow moves slowly in the microfluidic systems at the macroscopic point of view. There is number which helps to separate the laminar and the turbulent flow theoretically, and it is called Reynolds number. The Reynolds number is in the microfluidic systems so small that flow is usually assumed to be laminar. In the microfluidic systems the channel diameter is small and in this study it is also circular, but not always. The dimensions are in micrometers. The equation (Eq. 2.2) for the Reynolds number is seen below:

$$Re = \frac{\rho v D_H}{\mu} = \frac{v D_H}{\nu} = \frac{Q D_H}{\nu A} \tag{2.2}$$

where ρ is the fluid density, v is the mean velocity of the object relative to the fluid, D_H ($D_H = 4A/p$, A is cross-sectional area, p is wetted perimeter of cross section) is the hydraulic diameter of the channel, which is term for noncircular channel and it is used like diameter for circular channels, μ is the dynamic viscosity of the fluid, ν is the kinematic viscosity, Q is the volumetric flow rate and the A is the cross-sectional area of the channel. So several parameters and their functions are affected the Reynolds number. There is transitional area from $Re = 1500$ to $Re = 2500$ where flow has changed from laminar to turbulent, but usually in the case of the

channels is considered when $Re < 1500$, the flow is laminar and when $Re > 1500$, the flow is turbulent. [2, p. 39]

When the liquid flow is in the microfluidic channel, there are usually also gas bubbles. On the interface of the gas and the liquid there is certain contact line. In Fig. 2.4 is the contact line arrangement between the gas, the liquid and the solid, and the interfacial surface tensions γ_{sl}, γ_{lg} and γ_{sg} are the solid-liquid, the liquid-gas and the solid-gas, respectively. There is also the contact angle θ. When the angle is smaller than 90°, the liquid is said to *wet* the surface, and then the liquid is *hydrophilic*. When the angle is greater than 90°, the liquid is *nonwetting* and the liquid is *hydrophobic*. In Fig. 2.4 is also seen how the hydrophilic and the hydrophobic liquid behave on the surface and in the channel. [2, p. 46]

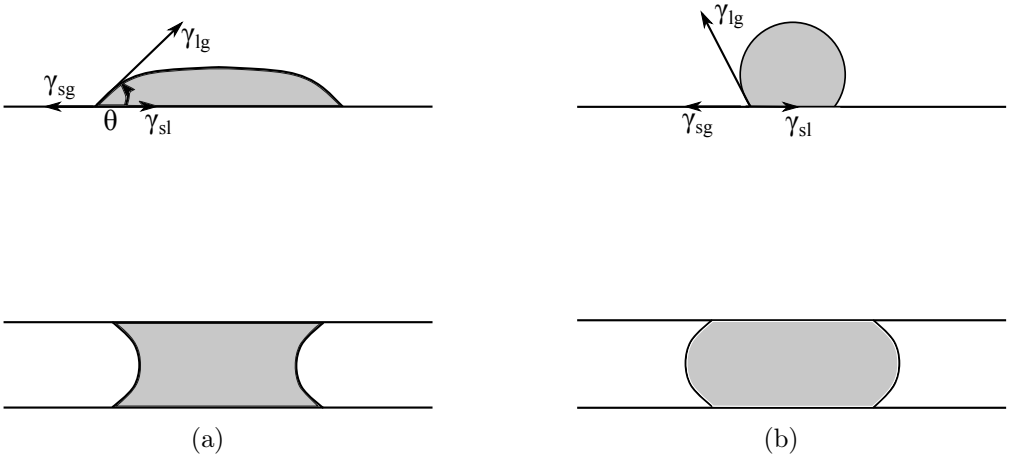


Figure 2.4. *Sketches of (a) hydrophilic and (b) hydrophobic liquids on the surface and in the channel. The interfacial tensions γ_{sl}, γ_{lg} and γ_{sg} and the contact angle θ between matter states. Adapted from [2, pp. 46; 6].*

Gas bubbles are important phenomenon in small channels. They can be avoided but it is good to understand the movement of the gas bubble in the microchannel. There is the interfacial surface tension γ_{lg}, so there is a pressure change across the liquid-gas interface. The change of pressure (Eq. 2.3) is happened in a capillary tube, which has a radius r:

$$\Delta p = \frac{2\gamma_{lg}\cos\theta}{r}. \tag{2.3}$$

This kind of gas bubble in the channel can be moved, when the pressure difference is:

$$\Delta p = \frac{2\gamma_g}{r} \tag{2.4}$$

where γ_g is a frictional surface parameter. For both equations above, the pressure

difference will increase when the channel radius is decreased. [2, p. 47; 7]

The gas bubbles and the liquid can be moved in the channels with help of pressure difference method, with electro-osmosis or with electrophoresis, for example. These methods with electricity are based on charge of molecules and how the charged particles move in the electric field. The pressure difference method is used in this study, and the pressure difference is created with a syringe pump. This kind of mechanical pump has benefits like high output pressure and a wide range of flow velocities. [3, 6]

2.1.3 Brownian motion and diffusion

As mentioned in Section 2.1, on the submicrometer scales the Brownian motion is the dominant way how the particles are transported. It can be considered as a random walk. Basic idea of 2D random walker is shown in Fig. 2.5.

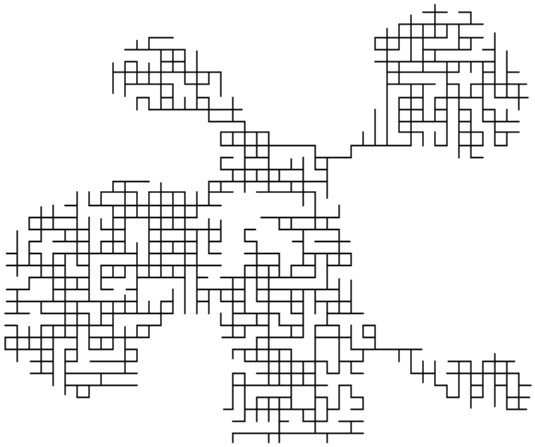


Figure 2.5. *Basic idea of the random walker. This is 2D random walker with specific step. Adapted from [8, p. 112].*

From this basic idea, Einstein has improved the idea in 1905. He found that if the average over numerous fluid-particle collisions is taken, then the average square displacement of the particle in a specific amount of time t is

$$< x^2 >= \frac{2k_bTt}{f} \tag{2.5}$$

where k_b is the Boltzmann's constant, T is the temperature and f is the friction coefficient. It can be assumed that the molecules are spherically packed, so the friction coefficient is

$$f = 6\pi\eta r \tag{2.6}$$

where η is the fluid viscosity and r is the radius of the molecule. Then the average square displacement is

$$< x^2 >= \frac{2tk_bT}{6\pi\eta r}. \tag{2.7}$$

With Eq. 2.7 and help of the fact of diffusion law (1D)

$$< x^2 >= 2Dt, \tag{2.8}$$

where D is the diffusion constant, can be ended to the Stokes-Einstein equation

$$D = \frac{k_bT}{6\pi\eta r}. \tag{2.9}$$

[9; 10 pp. 871–872; 8, pp. 115–120]

With this Stokes-Einstein equation can be explained the movement of particles. The movement happens from the high concentration to the low concentration. This is quite slow transport mechanism and happens always, but is more dominant in the microscale. When the velocity field of the liquid is zero, the diffusion is considered to be pure. In the microchannels, the diffusion is usually the dominant mixing method and often the slow step in a chemical process. Sometimes more effective mixing method is needed, but then the mixing is done actively. [3]

Also in the water treatment process the Brownian motion is taken place. The particles in the water move like random walkers and they collide. Because of the interaction between the particles, they form flocs (flocculation). In the flocculation the particles form larger clusters. This phenomenon is taken place after coagulation and it separates suspended solids from the water in the water treatment process. [11]

2.2 Water treatment process

The water treatment process is an important process for purpose of having clean drinking water. There are different kinds of particles in the water and they have different properties. Correct applications of coagulation and flocculation processes have to be selected and all the properties of the particles and interactions between the particles have to be considered. After the coagulation and the flocculation, sedimentation, filtration and disinfection are done.

In the water treatment process the raw water is mixed with the coagulants during the coagulation. The coagulants are chemical particles which have specific charge and they can neutralize the particles in raw water. The suspended particles stick together and they form microflocs. The coagulation is improved with mixing. From the chemical process (the coagulation) is moved to the physical process (floccula-

tion). The main task of the flocculation is to make bigger flocs. Then the water with the flocs is moved to the large tank where the sedimentation is happened. The most of the flocs settle out, but the smallest flocs and particles are filtered out from the raw water. After these steps there are still the bacteria and micro-organism in the water, so the disinfection like ozonization is needed. The last filtration is an active carbon filtering and it is the last filtering before the UV-light disinfection. After these water treatment steps the water can be pumped to the water chambers.

2.2.1 Coagulation

The first step of the process is the coagulation. The basic idea is shown in Fig. 2.6 Raw water is led to the chamber and different chemicals, like polymers, are blended to it. The water impurities are clustered with these chemicals to a little bit bigger particle groups. The used chemicals are called coagulants. [12]

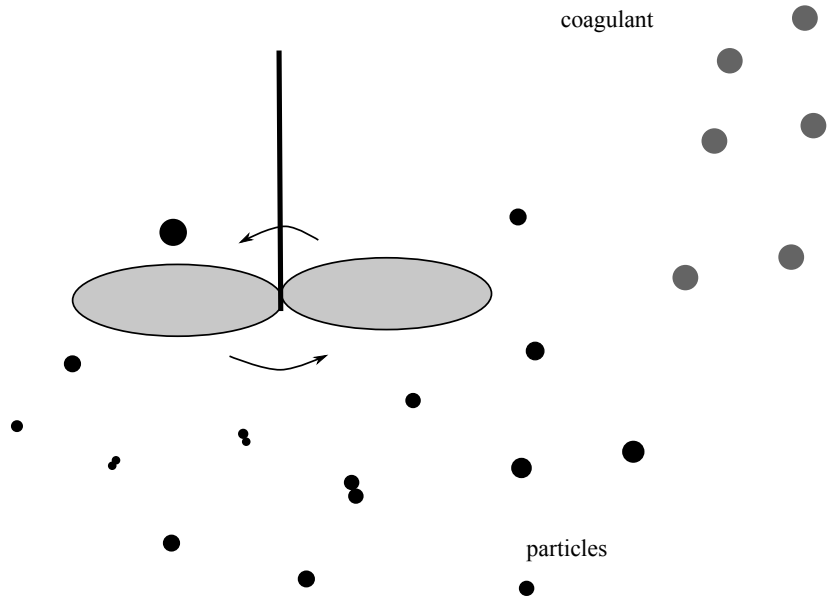


Figure 2.6. *Basic idea of the coagulation. Coagulant is added to the water and with mixing the particles collide, neutralization is happened and microflocs are formed.*

The particles have negative, positive or neutral charge. The coagulants with opposite charges than those particles are added to the water. The particles and the coagulants are affected by the Lennard-Jones -potential and the coagulants will neutralize the charges of the particles. During the charge neutralization, the suspended particles are able to stick together. Slightly larger particles which are formed with the neutralization process cannot be seen with naked eye. Usually more than one type (negative, positive or neutral charge) of coagulant is needed before all particles form microflocs. [12; 13, p.75]

A rapid-mix with a high energy is needed to promote particle collisions and to achieve a good coagulation. If some over-mixing is happened, it does not affect the coagulation but if there is not enough mixing the coagulation step is incomplete. Usually contact time of the coagulation process is one to three minutes.

After the coagulation (the chemical process), the flocculation (the physical process) is taken place. The main point in this process is that with gentle mixing the particle size is increased from the submicroscopic microfloc to the visible particles. [12]

2.2.2 Flocculation

The flocculation is process where particles aggregate and on the same time there is fragmentation. Flocculants can be anionic, cationic or neutral. The anionic flocculants is reacted against positively charged suspension; it absorbs particles and causes destabilization by bridging or by charge neutralization. This is happened under the acidic pH conditions. The cationic flocculants is reacted against the negatively charged and it happened under the basic pH conditions, respectively. When the net charge of the surface is zero, the pH is referred as the point of zero charge, and the particles can be stuck together. [11]

There is an equation for population balance which includes both aggregation and fragmentation. The equation (Eq. 2.10) is presented below.

$$\begin{aligned}\frac{\partial n(V_1,t)}{\partial t} = & - \int_0^\infty \alpha(V_1,V_2)\beta(V_1,V_2)n(V_2,t)dV_2 \\ & + \frac{1}{2}\int_0^{V_1} \alpha(V_1-V_2,V_2)\beta(V_1-V_2,V_2)n(V_1-V_2,t)dV_2 \\ & - S(V_1)n(V_1,t) + \int_{V_1}^\infty S(V_2)\gamma(V_1,V_2)n(V_2,t)dV_2, \end{aligned} \tag{2.10}$$

where n is the number of concentration of particles or aggregates, V_1 and V_2 are the particle or aggregate volumes, t is the flocculation time, α is the collision efficiency factor, β is the collision frequency factor, S is the specific rate constant and γ is the breakage distribution function. [11, p. 788]

This kind of flocculation which is caused by the Brownian motion is natural and it is called as perikinetic floc formation. The collision frequency factor for the perikinetic aggregation is given by

$$\beta_{i,j} = \frac{2k_BT}{3\eta}\left(\frac{1}{d_i}+\frac{1}{d_j}\right)(d_i+d_j), \tag{2.11}$$

where d_i and d_j are the collision diameters of aggregates belonging to sections i and

j. [11, p. 790]

There is also orthokinetic floc formation which is caused by gentle mixing. Careful attention to the mixing velocity and to the amount of mixing energy is required. The collision frequency factor for the orthokinetic aggregation is given by

$$\beta_{i,j} = \frac{G}{6}(d_i + d_j)^3, \tag{2.12}$$

where G is the average velocity gradient or the shear rate. [11, p. 790]

Basically the difference between the perikinetic and the orthokinetic flocculation is how those have been caused by. The perikinetic flocculation is kind of passive because there is always Brownian motion, but the orthokinetic is active because of the active mixing. Careful attention to the mixing is required in the flocculation process. If the flocs start to break because of too large mixing energy and too high mixing velocity, it is hard to get the flocs reform to their optimum size and strenght again. The flocculation step takes time from 20 to 45 minutes. The basic idea of the flocculation is shown in Fig. 2.7.

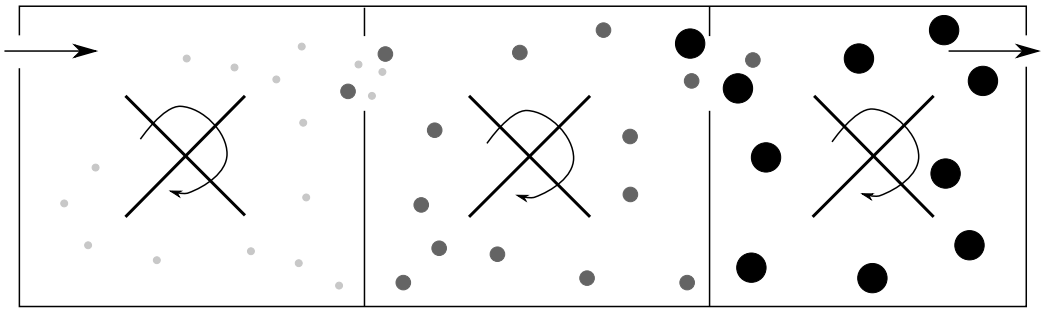


Figure 2.7. *Basic idea of the flocculation. The water is leaded throught the basins and with mixing the flocculation is happened gently with flocculation steps. The water with the macroflocs is entered into large tank when the largest flocs are settled to the bottom.*

With these flocculation methods described above the floc size is grown. When there is not mixing, the perikinetic flocculation takes place, and in the case of mixing, the orthokinetic flocculation takes place. The microflocs are grown first to the visible flocs which are called pinflocs. When macroflocs are formed and the floc has reached its optimum size and strength, the water is ready for the next process steps which are sedimentation, filtration and disinfection.

2.2.3 Sedimentation, filtration and disinfection

The sedimentation is the next step in the water treatment process. The water with the flocs is entered into the large tank where is slow flow. The largest flocs are settled to the bottom of the tank because of gravity and density between the solid flocs and the water. This step takes four hour in minimum. The time is depended

the size, especially the depth, of the tank. The deep tank allows more flocs to settle out and the larger flocs take smaller with them to the bottom when they are falling. Usually the smallest flocs are moved forward to the filtration because it takes too much time when all the flocs are settled down to the bottom of the tank. [12, 14]

After the sedimentation there are different filtrations and disinfections. In the filtration process the remaining particles and the unsettled flocs are filtered with different kinds of filtration systems. The last filtration is done through the active carbon filter where for example humus remains. [12, 14]

First disinfection is done after the first filtration. In this phase bacteria and micro-organisms are still in the water. Usually those are got away with ozonization. The smell and the taste of the water are also improved by the ozonization. In some point before the last filtration, carbon oxide is added to the water. The carbon oxide increases the basicity and this way corrosion is decreased. After the carbon filtering there is the last disinfection phase which is done with ultraviolet light. [12, 14]

After all this phases in the water process, the water is clean and ready to be pumped with the high pressure to the water chambers. During the process is important that the quality of the water can be tested easily, quickly and faithfully. [12, 14]

2.3 Water analysis in microfluidic systems

The testing method which is easy and quick, can be created with help of microfluidics. Microfluidics can affect the chemical analysis, like microchips have revolutionized computers and electronics. One of the main advantages of the microfluidic systems is speed. For example electrophoresis is 100 times faster when the system is ten times smaller. Micro-scale has made possible to integrate chemistry with mechanics, electronics and optics, and several analytical systems are integrated into very small areas. [15]

According European Commission, the Danish water instrumentation specialist Danfoss, had project called MicroChem from 9/1998 to 10/2001 which goal was to produce a new micro-analysis system for monitoring the levels of chemical species. Basic requirements for this system have been measurement of concentrations of small ions, *in situ* measurements in the waste water treatment plants, measurements on drinking water and project provided new knowledge on micro system durability and reliability. [15, 16]

The project has given encouraging results and the MST has taken a major step forward. And of course there are few general benefits like reduced consumption of analyte chemicals because of the small volumes and the response times have been decreased because of small dimensions. One huge advantage is that more than one ion in the one system can be measured. [16]

On last decades the microfluidic applications are also developed to environmental analysis or environmentally related species. One benefit of small sample volume in the environmental analysis is the minimized risk of contamination. Also the small sample volume and fast results negate the transportation and the analysis can be made where the sample is detected. [17]

Different applications in miniaturized systems are developed to detect for example toxic metal ions, organic peroxides and other inorganic and organic pollutants. These pollutants can be atmospheric and water-based, and microfluidic systems for both circumstances are developed. [17]

Some applications have integrated cleanup and enrichment systems. These operations are usually needed because of the low concentrations. Environmental samples have a complex matrix with many components. In Table 2.1 below is shown different applications what are used in the microfluidic environmental analysis. [17]

Table 2.1. *Analysis methods and detection techniques of real samples. Adapted from [17].*

analysis	**detection technique**	**real sample**	**analyte**	**detection limit**	**reference**
MCE	absorbance	water	Cd^{2+}	6 µg/l	[18]
MCE	AFS	river water	As(III)	76 µg/l	[19]
µFIA	SERS	water	malachite green	1 ppb	[20]
µFIA	fluorescence	air	NO_2	10 ppb	[21]
SPE	-	diesel exhaust particles	benzo[a]pyrene	-	[22]
MCE	amperometric detection	ground water	TNT	24 ±1mg/l	[23]
µFIA	fluorescence	ambient air	SO_2	2.8 ppb	[24]

Microchip electrophoresis (MCE) is based on separations and has been successful analysis method in environmental fields. Used with absorbance as detection method, the MCE can be used to detect toxic metal cations from water, like Cd^{2+}. The MCE can be also used with atomic fluorescence spectrometry (AFS) and amperometric detection. The AFS is powerful detector for environmental ionic pollutants, like for As(III) which is detected from river water. A "tube-in-tube" interface is designed to the couple of the MCE devices to the AFS. This kind of coupled system allows the rapid measurement of inorganic arsenic. The amperometric detection is typically used in the MCE systems to analyse phenolic compounds. Trinitrotoluene (TNT) and other nitroaromatic explosives can be separated with amperometric detection from ground water and soil extracts. From Table 2.1 can be seen that the MCE analyses are done from different water samples, but no gas samples. [17–19, 23]

Microflow injection analysis (µFIA) can be also used with different detection techniques, for example surface-enhanced Raman spectroscopy (SERS) and fluorescence.

It can be used to analyse both water and gas samples. Application, where is zigzag-shaped microfluidic channel on PDMS chip with SERS, is designed to detect the industrial dye malachite green. These malachite green molecules are absorbed onto silver nanoparticles more effectively along the zigzag-channel. The fluorescence is used as a detection technique in gas sensors which are used to measure atmospheric pollutants, like NO_2 and SO_2. [17, 20, 21, 24]

Solid phase extraction (SPE) is used to on-chip cleanup of benzo[a]pyrene and benzo[k]fluoranthene from diesel exhaust particles. This is done with silica gel beads which are in the microchannel. [17, 22]

Anlysis methods and detection techniques which are mentioned above, are developed over five years ago and they are stated out good and fuctional. In last five years the development is been also hot topic and new methods and techniques are studied. Few of them are introduced below.

One major analyte is mercury and its ion Hg^2+ which can be found for example from water. Mercury is heavymetal pollutant, toxic and it should be concidered as bioaccumulate matter. Environmental analysis of mercury is usually done with atomic absorbtion spectroscopy, atomic fluorescence and plasma-mass spectroscopy. These are sensitive and accurate, but the are expensive, take time and they need pretreatment. One effective and quite cheap alternative is combination of microfluidic immunoassay and SPE. An antibody for the immunoassay is group of mercury-organic compounds (EDTA, glutathione, 6-mercaptonicotinic acid). With this kind of methods the analyte can be detected from tap water, mineral water, actual river water and highly contaminated mockup river water. [25]

From surface water can be determined toxin, pharmaceutical and endocrine disrupting compounds (EDC) with high performance extraction disk cartridge. A high performance extraction disk cartridge preconcentrates the analytes. This is one method what can be used with fluorescence detection. To analyse the extracts is used a confocal laser which induce fluorescence detection setup. [26]

Also from surface water can be detected Cr(VI) with optical methods. For detection is used integration of light emitting diodes and photodiodes to perform a colorimetric analysis. Determination is based on the diphenylcarbazide reagent. This kind of simple absorbance measurement can be integrated in microfluidic system. This kind of measurement system can be used to test surface water near industry plants because Cr(VI) is generally produced by industrial processes. [27]

Perchlorate, nitroaromatic compounds and gold (Au(III)) and iron (Fe(III)) ions can be detected from drinking and waste water. Those can be detected with help of contact conductivity, carbon disk electrode and paper-based electrochemical device, respectively. Perchlorate is detected electrophoresis device with contact conductivity detection; one new MCE detection method. Nitroaromatic compounds are detected

with amperometric detection as mentioned earlier. This carbon disk electrode is used to detection. This kind of paper-based devices are low-cost, simple, portable and disposable. Other paper-based device is this kind of electrochemical device which combines electrochemical and colorimetric detection. This system can detect gold and iron ions. [28–30]

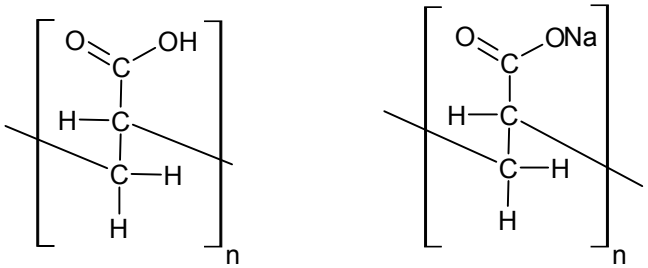
Clear trend seems to be the production for non-techinical instrumentarion and easy-to-use devices for minimally trained technicians. One major thing is portability which is still selling point of microfluidics. Microchip electrophoresis is one most used analysis method, but there are also optical and spectroscopic detection methods which are developed during last five years. [31]

2.4 Polymers and europium label

In the water treatment process, polymers are used as coagulants. Polymers are macromolecules which are consisted of monomers. In this project the monomers are acrylic acid and maleic acid. When these are polymerized, polyacrylic and polymaleic acid are formed. These polymers have also neutral sodium salts. When these sodium salts are polymerized, co-polymer of acrylic and maleic acid is formed. These polymers are linear and quite simple. Polymers have different properties and a length of polymers varies. The length depends how many monomers are polymerized. In next few sections properties are explained more accurately.

2.4.1 Polyacrylic and polymaleic acid and their sodium salts

Polyacrylic acid (PAA) is an anionic polymer. Structure of the PAA is seen in Fig. 2.8. In the water of neutral pH, the protons of the PAA sidechains are lost and polymer will have negative charge. The charge is turned to neutral, when sodium is added.



(a) (b)

Figure 2.8. *Sketches of (a) polyacrylic acid and (b) its sodium salt (sodium polyacrelate).*

The sodium salt of polyacrylic acid is called sodium polyacrylate and also as waterlock. Because sodium neutralize polyacrylates, it is most commonly used in industry. Also other salts are used such as potassium. It is used in many consumer

products. It can absorb water as much as 200 to 300 times its mass. One example of the applications, where super absorbent polymers are used, is baby diapers.

The polyacrylic acid and its natrium salt are both in liquid form, colour of them is pale and there is not smell or it is very light (for sodium polyacrylate). Both of these are totally soluble in water and they are not fat-soluble. The polyacrylic acid has pH around 2 and sodium polyacrylate is neutral (pH 7-8). When sodium is added to the polyacrylic acid, the density is increased and the dynamic viscosity is decreased. [32, 33]

Maleic acid has its carboxyl groups so that when the water molecule is lost, a cyclic anhydride is formed. The polymer is formed from this anhydride and polymaleic acid is known as hydrolyzed polymaleic anhydride (HPMA). The structure is shown in Fig. 2.9. From the figure can be seen that in the polymer every other maleic acid is in anhydrized form and every other is in the normal form. The average molecular weight is from 400 to 800. It is nontoxic, has good solubility in water and it has high chemical and thermal stability. Together with zinc salts it can inhibit carbon steel corrosion. [34, p. 309]

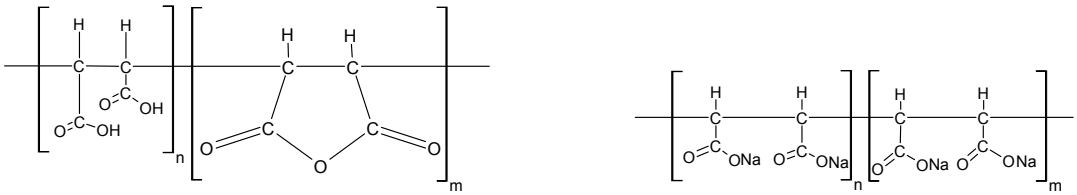


Figure 2.9. *Sketches of (a) hydrolysed polymaleic anhydride and (b) its sodium salt.*

In the structure of the sodium salt of polymaleic acid, the polymaleic acid is not in the form of the hydrolyzed anhydride. The hydrogens of alcohol groups are replaced by sodium atoms and polymer is neutralized.

2.4.2 Copolymer

Polymers above are homopolymers which are made from single monomer. When the mixtures of these monomers are polymerized, copolymers are compounded. The copolymer can be alternating, random, block or graft. How the monomers are arranged is coincidental and amount of possibilities is unlimited. With a free-radical chain-growth process is polymerized alternating and random copolymers; for block or graft copolymers the special methods are needed. [34, p. 422]

The copolymer (Fig. 2.10) of the sodium salts of acrylic acid and maleic acid are formed in this research. Because of sodium, the copolymer is also quite neutral (pH 8). The group on the end of the polymer is called thiol group. The copolymer solution is light yellow and it hardly smell. It is also water soluble. The density of

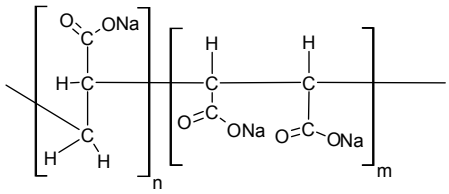


Figure 2.10. *The copolymer of sodium salts of acrylic acid and maleic acid.*

the copolymer is same magnitude as the density of sodium polyacrylate, also the dynamic viscosity is same order than the dynamic viscosity of the sodium polyacrylate. [35]

The europium chelate is connected to the copolymer at University of Turku. There are some different possibilities where to connect the chelate, but carboxyl groups of the polymers seem to be best alternatives in this moment.

2.4.3 Europium label

Europium has atomic number 63 and it belongs to the series of lanthanide. Its oxidation state is +3 like other members of the series. It does not have special role in biology and it is not toxic like other heavy metals. It even does not harm environment. [36]

Europium is the most reactive rare earth element and it reacts with water like calcium. Also oxidization is rapid process so it will not stay shiny in the air at room temperature. Neutrons are absorbed by europium and that is why it is used in nuclear reactor control rods. The phenomenon of fluorescence of the europium is usually used. [36]

In this project, the europium is form of Eu-chelate which is connected to the carboxylic groups of the copolymer. One example of structure of a Eu(III) chelate as a label is shown in Fig. 2.11. In the figure can be seen how the chelation is done at the metal binding site by nitrogen and carboxyl groups. [37]

This copolymer-chelate -compound can be seen in the ultraviolet light (UV-light) because the fluorescence which compound is emitted. The chelate is compound where metal ion is bound to the chelator. The chelator is an organic aromatic chromophore where is several metal-binding groups, like nitrogens and carboxyls. This kind of chelators (one or more) can be bound to the metal ion and the entire fluorescent chelate is composed. [37]

The lanthanides have large Stokes' shift. This allows the wavelength filtering against the nonspecific background signal, and other luminescent species. Other advantages of the lanthanides are the narrow-band emission and the long luminescence lifetime (from microsecond to millisecond). Of course there are same disadvantages

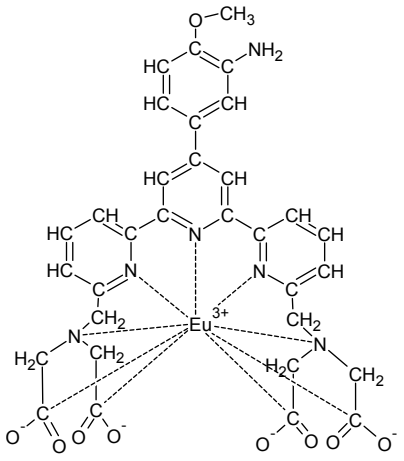


Figure 2.11. *The example of Eu-chelate. Adapted from [37].*

like metal-complex may be low (special handling to chelate binding), and relatively low fluorescence yield compared with the best organic fluorophores. [37]

This chelate example is also an example of europium-chelate -compound which can be bind directly to the protein, for example. The europium-chelate -compound is considered as probe for determination of binding and hybridization. This kind of compound has high molar absorptivity. The Eu^{3+} is bound with all nine coordinate bond to the chelate and the chelate keeps the europium ion under most conditions. [38]

2.5 Fluorescence measurement

2.5.1 Fluorescence

Fluorescence is a form of luminescence which is an emission of light. The fluorescence is a result of absorption of photons. The emitted photons have lower energy level than absorbed ones, because of the energy lost to vibrations. In this work the europium chelates are used and fluorescence spectroscopy is time-resolved. [37]

The photons are absorbed into the europium. The absorbed photons have certain wavelength and energy. For example in this study the wavelength is on the area of the UV-light. The energy of absorbed photon excites the ground state electron and the electron is excited to the higher orbital level which is called the first excited state. When the excitation is relaxed, usually after 1-10 nanoseconds, the electron returns to the ground state and the energy and heat are emitted. The emitted energy of photon is smaller (longer wavelength) than the energy of the absorbed photon. The excitation and emission are shown in Fig. 2.12. The energy of emitted photon is detected by fluorescence spectroscopy. [37]

Fluorescence is measured by fluorescence spectroscopy (fluorometry, spectrofluo-

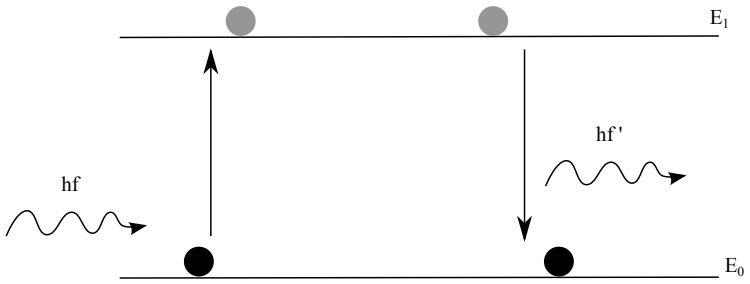


Figure 2.12. *The light photon (hf) is absorbed and the electron is excited. Exitation is relaxed, the electron is returned to the ground state and photon is emitted with energy of hf'.*

rometry) which is a type of electromagnetic spectroscopy. The excited light is kept at constant wavelength and different frequencies of fluorescent light are measured.

2.5.2 Time-resolved fluorescence

Time-resolved spectroscopy is an extension of normal fluorescence spectroscopy. The fluorescence is here detected as function of time after excitation by a flash of light. There are different ways to obtain the time resolution; it depends on the wanted sensitivity and time resolution. In the time-resolved fluorescence (TRF) the luminescence from long-lived species is detected after delay. During the delay, the short-lived species are decayed. [37]

The time-resolved fluorometric system can be performed when the label which is used has enough long-lived luminescent. In the biological samples typical background emission is caused by organic fluorophores and it is short-lived. Emission lifetime for long-lived label is from microseconds to milliseconds. [37]

The chelate offers good emission characteristics because the combination of organic chromophore portion. This portion is made of chelate and lanthanide ion. The chelate absorbs the excitation light and the ion accepts the energy and emits it at longer wavelengths. This lanthanide ion luminescence has usually the width of the individual transitions from 1 to 20 nm. This kind of large shift (Stokes' shift) allows wavelength filtering against background signals. There are also some disadvantages, such as stability of the metal-complex might be low and the fluorescence yield is relatively low when compared with the best organic fluorophores. [37]

2.5.3 Measurement instrumentation

The instrumentation consists of a pulsed or time-gated light source, wavelength filtering, holder for the sample, wavelength filter for the emitted light (monochromator) and time-gated detector. This kind of simplification of the measurement instrumentation is shown in Fig. 2.13. The source of light has certain energy, so the

photons have specific range of wavelengths. The wavelength filtration only transmits the suitable wavelength, for example wavelength from ultraviolet range. The light is channeled to the sample compartment and the light is absorbed to the sample. Electrons of atoms of the sample are excited. When excitation is relaxed the sample emits photon with lower energy. This photon beam goes through the monochromator. In our equipment the monochromator is actually a filter. The photon beam is travelled in to the monochromator and it is reflected by the concave mirror. From there the photon beam goes through prism or grid and different wavelenghts are separated, and unwanted wavelengths are reflected away, for example. Then the photon beam is reflected again by the concave mirror and it is travelled through output to the photomultiplier tube (PMT). [37, 39]

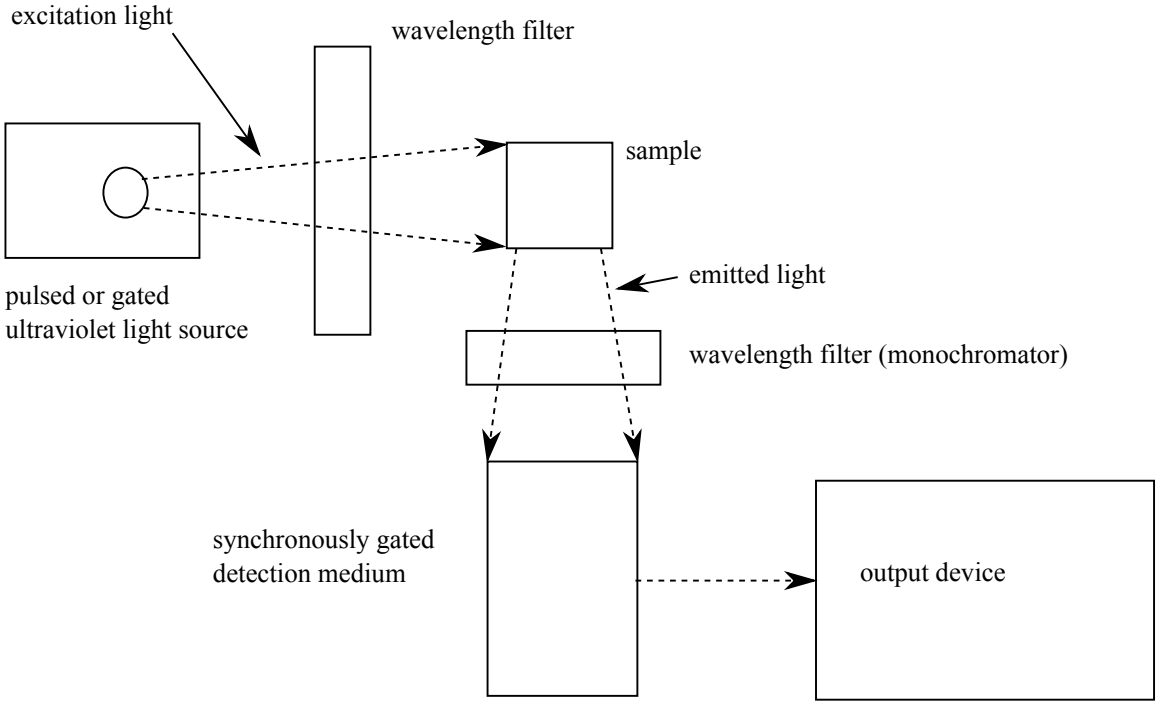


Figure 2.13. *Simplified measurement instrumentation. Adapted from [37]*

The beam is detected as single photons on the PMT. With this PMT can be measured very weak scintillations, because on the outer surface is a light cathode which is coated with light sensitive material. When photon is absorbed to the material the electron is emitted if energy of photon is larger than work function of electron. Amount of electrons is commensurate to the amount of photons, but the amount of electrons has to increase with amplifier stages. Every amplifier stage is on greater potential than previous one. The amount of electrons which arrive to the last anode is $10^5 - 10^8$ times greater than amount of electrons which have emitted from light cathode. Thus the amplification, the amount of amplified electrons is still commensurate to the amount of absorbed photons. Efficiency of the fluorescence

amplification process can be defined as a ratio of number of emitted and absorbed photons, and it is called the fluorescence quantum yield. The quantum yield can be shown with Eq. 2.13.

$$\Phi = \frac{Number\ of\ emitted\ photons}{Number\ of\ absorbed\ photons} = \frac{k_f}{\sum_i k_i} \tag{2.13}$$

where k_f is the rate of spontaneous emission of radiation and $\sum_i k_i$ is the sum of all rates of excited state decay. For best situation the yield is one emitted photon per one absorbed photon. [37, 40]

3. RESEARCH EQUIPMENT, MATERIALS AND METHODS

This chapter is divided to two parts. The first part is for the test set up, which includes introduction of the measurement equipment, other measurement parts and the reagents which are used. The second part introduces the measurement methods. In this part is get familiarized how the measurements are done.

3.1 Test set up

3.1.1 Microfluidic cartridge

The microfluidic system is in this study microfluidic cartridge. It is quite simple; there are chamber and two channels, the inlet and the outlet. This is shown in Fig. 3.1.

Previously, the microfluidic systems have been made of silicon, metal or glass. Nowadays, polymers are preferred in biological applications. In this study, the cartridge is made on polystyrene (PS) which is used because of its low cost and it is easy to process. Also poly(methyl methacrylate) (PMMA) is tested but the

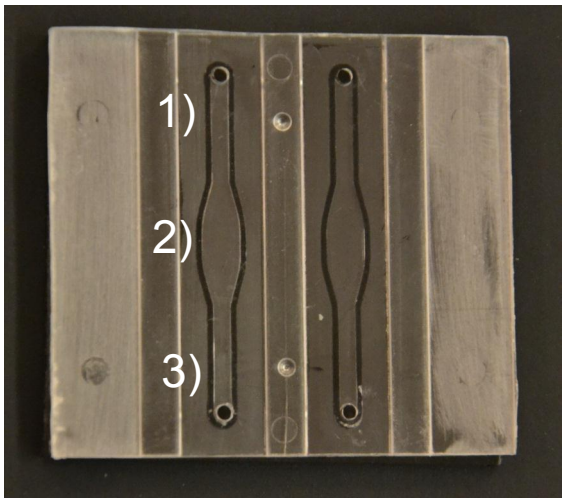


Figure 3.1. *On the cartridge is two sides which are identical. There is 1) inlet with hole and channel, which leads to chamber. Then 2) the actual measurement chamber, where the measurement is done. 3) The outlet channel and hole, which leads out from the chamber.*

laser welding does not work correctly. Some benefits with the PS are also that it is transparent, durable, nontoxic and it has good mechanical properties. Nevertheless, there are also disadvantages, such as poor chemical resistance and hydrophobicity. But these disadvantages can be improved with different techniques.

This kind of cartridge, which has only the measurement chamber is enough to research the needed phenomena. Usually the cartridges have also other chambers and channels, and they look more complex than this one which is used. Anyway, the measurement is performed from the chamber as scanning, or the measurement point is fixed in the middle of the chamber. In this cartridge, the chamber has the volume of 27 µl, but with inlet and outlet the volume is about 50 µl. The height of the chamber is 0.5 mm.

The cartridge is fabricated by injection molding at TUT in polymer laboratory of Department of Material Science. The external dimensions of the cartridge are 55 mm × 45 mm × 2 mm. There is also a cover for the cartridge and it is black or transparent PS plate. In this study, the used cover plate is black. The sealing is performed by laser and laser welding is done by Department of Production Engineering at TUT. Before testing the cartridge is washed and rinsed with isopropanol and deionized (DI) water, respectively.

3.1.2 Measurement equipment

The measurements are done with two measurement equipment. The steady state measurements are done with Wallac 1420-018 multilabel counter (PerkinElmer), re-

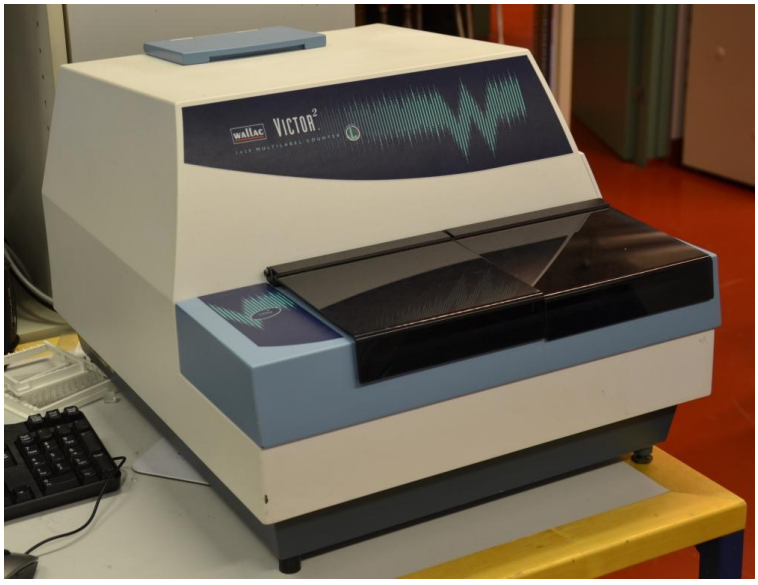


Figure 3.2. *Victor^2 (Wallac 1420-018, PerkinElmer) is multilabel counter which uses time-resolved fluorescence for europium detection.*

ferred as Victor^2 (Fig. 3.2). Equipment is modified so that it is only suitable for TRF for europium detection. Otherwise the devise is for quantitative detection of light emitting or light absorbing markers, and this type of devise is also suitable for flash or glow luminometry, UV absorbance, photometry and fluorescence polarization. The UV xenon flash lamp (spectral range 230-400), which is used to the TRF, produces the excitation of electrons. Then the excited photons travel through the emission filter. In this study is used the filter for europium (615 nm). [41]

The measurement protocol for Victor^2 is designed so that at first the cartridge plate is installed to the plate adapter. The distances from the horizontal and from the vertical edges to the middle point of the measurement chamber are measured. The distances are saved to the plate program. The measurement type is also selected. Now the measurement is done ten times from this one certain point.

The flow measurement is done with equipment (Fig. 3.3) which is modified from old CHAMELEON ™equipment of Hidex (referred as modified Hidex equipment). The source of light is xenon flash light and it is used to excite the sample. The sample is excited (wavelength from 190 nm to 900 nm) and it emits (wavelength from 360 nm to 850 nm) fluorescence photons. In this study the used excitation wavelength is 340 nm and photons, which are emitted, have wavelength of 616 nm. The emitted photons are travelled through monochromator (Eu-filter) to the PMT, where photons are detected as single events. Then signal is moved to the photon counter which processes the signal. [42, 43]

This modified Hidex equipment is used for flow measurements because the flow is easier to create in this equipment than inside the Victor^2. During the initial measurements was noticed, that this modified Hidex equipment does not give as high signal levels than Victor^2. The variation was not as good as with Victor^2, but good enough to study the measurement with flow.

3.1.3 Modifications for Hidex equipment

The modifications needed for the Hidex equipment are performed to find out the measurement point and develop an adapter system, which fix the cartridge on right place, and the holes for the tubing is drilled.

First things to do with the modified Hidex equipment are to find out where is the location of the light beam. Originally there is a carrier which is moving the multiwell plate. There are several wells on the plate and the original carrier moves during the measurement when the measurement is proceed from the well to another. The carrier is not needed now and it is fixed on the specific place, which is against the corner of back and right wall. An adapter is done for the cartridge and with help of it the place of the cartridge is fixed on horizontal and vertical dimensions.

The adapter is quite simple. The model and picture is shown in Fig. 3.4. The

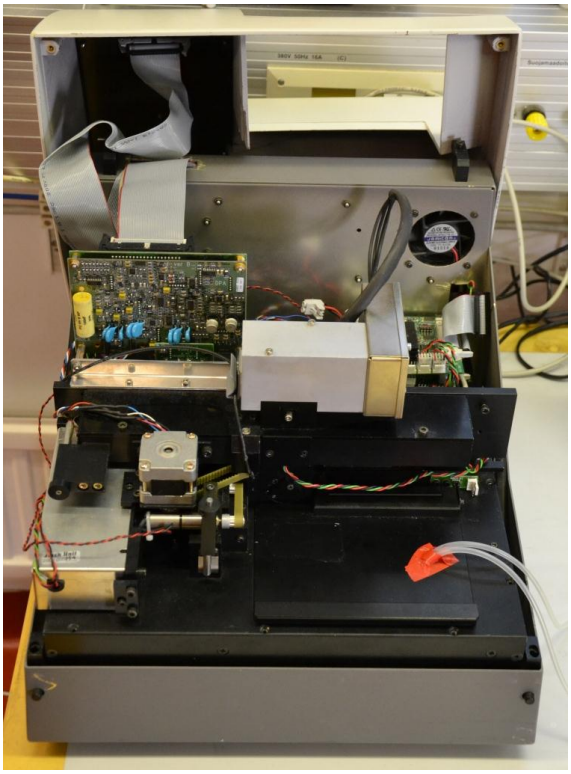


Figure 3.3. *The modified Hidex equipment is used to measure time-resolved fluorescence when the sample is moving in the measurement chamber.*

hole is because of bracket on the outer surface of the original carrier. There is also little bracket on the bottom of the adapter, which is used to fix the adapter on its place. The adapter bracket is fitted to the gap, which is on the plate under the carrier. The cartridge is initially fixed with double-sided tape and with separated plastic plates on the adapter.

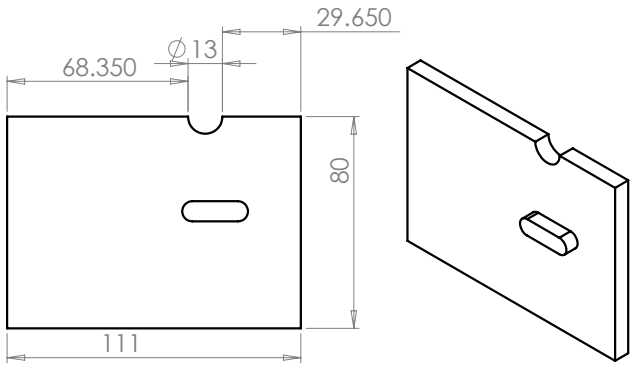


Figure 3.4. *The adapter for the cartridge.*

The cartridge is fixed so on the adapter that the inlet is near of the back wall and the outlet is closer of the front wall of the equipment. Good place for the light spot is found when the distance from the back edge of the adapter to the hole of

the inlet is 14 mm, and the distance from the left edge of the adapter to the hole of the inlet is 19 mm. In Fig. 3.5 is shown the adapter, the cartridge and the tubes on their place.

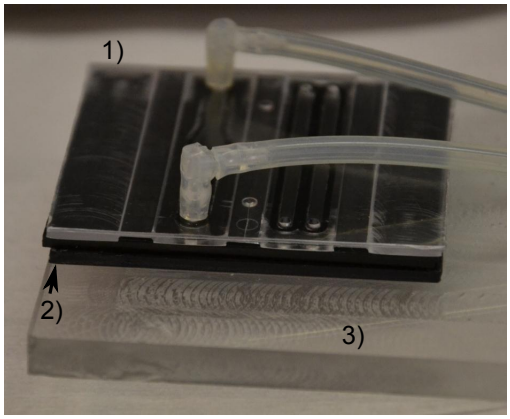


Figure 3.5. *On the figure is 1) cartridge on the top of 2) extra plate. Below the extra plate is 3) the adapter. The tubes are connected to the inlet and outlet holes on the top of the cartridge.*

The sample is pumped in the cartridge through the tubes. Those tubes are connected to the inlet and outlet. Connections between the cartridge and the tubes are done with connectors and tips of pipette. The connections are little bit problematic, because the loss of space above the cartridge and the tiny parts of connections. The tube-chip -system should be leak-proof so that the pump is able to suck the sample through the pipe system and into the chamber. The connector system is secured with the short stretch of silicone tube.

3.1.4 Injection molding and laser welding

Injection molding is one of manufacturing process which can be used when producing plastic parts. Advantages of the injection molding are for example production yield, minimal requirements for postmolding operations and also complex geometries can be produced. The mold itself must be well designed, because it is quite expensive to manufacture and it is used in mass production. [44]

The mold which is used in this study is designed in the research group of Micro- and Nanosystems in the department of Automation Science and Engineering. The injection molding is done in the Polymer laboratory of Material Science at TUT.

In the beginning of injection molding process, plastic granules are heated and homogenized. When the plastic granules are melted, the plastic mass is leaded with pressure to relatively cold mold. In the mold the plastic solidify and takes the shape of the mold. [44]

Laser welding is rapid technique which can be used to seal cartridges. The technique is also suitable for mass production because of its low unit cost. This sealing method can be also used because it is studied that signal levels are not affected of polystyrene cover on immunoassay measurements. [45]

By laser welding the sealing of the cartridge is get pressure-tight seal and the physical parameters or dimensions are not changed. During the sealing, the temperature rises but the stress amount into joining materials is reduced because the energy production is localized in laser welding. [45]

These two joining parts are pressed together and the laser beam passes through the transparent polymer (injection molded cartridge) and is absorbed by the other polymer (black cover). In that point optical energy of the laser is transformed into heat. The heat softens both polymer layers of the interface and the two molten materials form a mixture. The mixture of molten polymers forms an accurate local bond. In the welding technique the laser beam travels once through the welding path. [45]

3.1.5 Flow control devices and components

The flow can be made with help of syringe pump (New Era Pump Systems, Inc., USA). This mechanical pump (Fig. 3.6) is one of the simplest pumps used in microfluidics, but it is good enough to create suck in tubes. A stepper motor is used to move the plunger in the syringe. The pump takes 400 motor steps per revolution and 1/8 to 1/2 microsteps depending on motor speed. One pump can be used for a wide range of volumes and flow rates. With this kind of pump the dimensions of pump are larger and it is not integrated on the disposable device. [4, 46]

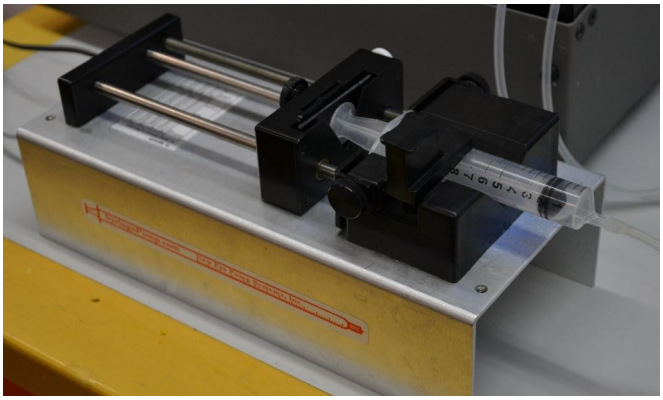


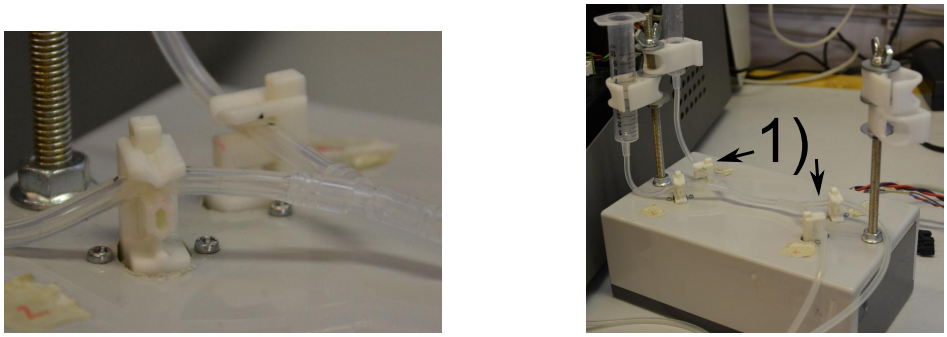
Figure 3.6. *The syringe pump is the last part of the system where all the waste is collected.*

The syringe up to 60 cc (1 cc = 1000 µl) can be used, but now the syringe size is 10 cc. Speed can be between 0.004205 cm/hr and 5.1005 cm/min and pumping rate

from 1699 ml/hr (B-D 60 cc syringe) to 0.73 µl/hr (B-D 1 cc syringe). The pump is used to move the liquids into and out from the measurement chamber. The 10 cc syringes are used, and then the withdraw rate can be from 6.876 µl/hr to 500.4 ml/hr. The pump is used with computer software. With the software the infusion speed is defined. The pump can be also programmed for other modes. [4, 46]

The syringe pump is connected with silicone tube to the outlet of the cartridge. In this study the syringe pump is connected as same way in every measurement. The inlet of the cartridge is connected with another tube which split up and ends to the washing solution container and to the process chamber. With withdraw option the pump sucks the fluids through the measurement chamber to the syringe. The connectors are made of tips of pipettes and tube connectors (3/32", Barbed Elbow, World Precision Instruments), which have to be leak-proof to generate the vacuum by the syringe pump.

The different parts are connected together with the tubing. The tubes are silicone tubes with inner diameter of 2 mm and outer diameter of 4 mm (VWR 228-0704). The process and the washing solution chamber are connected to the main tube with Y-connector (3/32", Barbed Tubing Assortment, World Precision Instruments) and from this main tube, the cartridge and bypass of the cartridge are separated with another Y-connector. There is one valve for each of these four branches.



(a) (b)

Figure 3.7. *Pictures of the valves and tubing. In the picture (a) are two valves and here can be seen that when the valve is closed, the middle part of the valve rises and squeezes the tube to stop the flow. In a picture (b) are the valves (1) and tubing on the side.*

The valves are simple and they can be open or close. When the valve is close, it squeezes the tube and the flow is stopped despite of the withdraw of the pump. The flow continues in the tube again when the valve is opened. The valve system is made in MST laboratory at TUT.

In this study, the flow stays laminar as usually in microfluidic systems. The main part of fluid is DI-water, so the fluid parameters of water ($\rho = 1000kg/m^3$, $\mu = 1{,}002 \cdot 10^{-3}$ kg/m ·s)can be used when Reynolds number is determined. The perimeter of inlet and outlet channel is 5 mm, in the silicone tube it is over 6 mm

and in the chamber it is 9 mm. The flow rate is 500 ml/hr in maximum during the washing. The Reynolds number in inlet/outlet channel, in the silicone tube and in the measurement chamber is about 110, 88 and 60, respectively. When the flow rate is decreased the Reynolds number is also decreased, and during the flow measurement the Reynolds numbers are below 0,3 (v = 1.25 ml/hr).

3.1.6 Reagents used in the research

In this study is used the Eu-labeled polymer (20 w-% = 20 g/100 ml), which is polymaleic acid derivative (HT III/7). The Eu-label is added on the synthesis phase of polymer. It is diluted with dialyse buffer (100 nM Tris-HCl, 20 mM NaCl, pH 7,75), referred later as "buffer". This buffer is used also as washing solution. The dilution series is done, with multiplier of five. In Table 3.1 can be seen the concentrations of the dilutions.

Table 3.1. *The concentrations of dilution series.*

concentration ($\mu g/ml$)	ratio
1000	1:200
200	1:1000
40	1:5000
8	1:25000
1.6	1:125000
0.32	1:625000

Comparison measurements are performed before the measurement. These measurements are done with the known Eu-STD solution (100 nM, gives 100 000 000 counts) which is diluted to the developmental solution. With this solution, the Eu particles are activated and the measurement is succeeded. The measurement equipment can detect about 1 000 000 counts, so the dilution is made 1:100 which gives 1 000 000 counts (1 nM). This known sample is measured with the both measurement equipment.

3.2 Measurement methods

This section introduces the measurements which are done during the research. There is several experiments which are performed to study the usability of microfluidic cartridges and Eu-labeled polymers in detecting polymer remains in waste water samples. The research questions are as follows: i) are the results repeatable between cartridges, ii) can different concentrations be measured and what is the detection limit, iii) can the same cartridge be used several times, if the cartridge is washed

between the measurements, and iv) can the measurements be performed using a flowing sample. The experiments are categorized accordingly.

The rest of the section is organized as follows. First, the experiments are performed to get familiar with the devices and material and to calibrate the measurements are described. Then is studied cartridge repeatability and the washing protocol. After that, concentration measurements are done with different sample concentrations. Also the detection limit is studied in this point. Then, the experimets are performed to find out is cartridge reusable when the washing is done between the measurements. At last is studied the measurement with flow.

3.2.1 Device familiarization and initial comparison

Before the actual measurements, the modified Hidex equipment is tested. This is done to figure out the basic behavior of the equipment and get familiar with it. During the familiarization, the influence of the height of the sample is tested. The measurement is done on two different heights and with the largest concentration. Then the device comparison is done. The comparison is done to see signal level of known sample. This is done with both equipment and also with the 96-plate to see the difference between the equipment and measurement platforms. Also different measurement locations on the area of measurement chamber are determined.

The influence of measurement height is performed using the following protocol. The sample ($c = 1000$ µg/ml, $V = 50$ µl) is pipetted through the inlet in the chamber while the cartridge is located outside the measurement equipment. The volume of 50 µl fills the entire chamber and the inlet and outlet channels, and this way is made sure that there is not air in the measurement chamber. The cartridge is placed into the adapter and they are placed in the modified Hidex equipment. The height is 9 mm (from the bottom edge of the adapter to the bottom of the measurement chamber) when the cartridge is right on the adapter. The measurement is done and repeated with the height of 11 mm (one cover between the adapter and the cartridge).

The height, where the cartridge is placed, affects the sharpness and size of light spot. Those properties affect the effectiveness of the excitation of europium of the sample. On the height of 11 mm (7000-8000 counts), the sample excitation is more effective and the signal is better than on the height of 9 mm (4000-5000 counts). The height cannot be increased more from the 11 mm because the tubes and connections take the rest of the empty space above the cartridge.

Comparison experiments are performed using a cartridge and a 96-well plate with the both equipment. 96-well is a standard measurement format and it is used for bio measurements for example in spectrophotometric measurements. When the sample is in the 96-well plate, the excitation light goes straight to the sample, but when

the sample is in the cartridge, the ecxitation light goes first through the transparent plastic.

Eu-STD solution (V = 50 µl) is pipetted in the measurement chamber and signal counts are measured first with the modified Hidex equipment and then with Victor^2. This kind of measurement order is used because the measurement time of Victor^2 is longer. After the cartridge measurement, the measurement is also done with the 96-well plate. The sample (V = 200 µl, as said in Victor^2 operation manual) is pipetted in the well and the order in using the devices is same.

3.2.2 Repeatability between cartridges and measurement locations, and washing protocol

The first actual research question is that are the measurements repeatable between parallel cartridges. This study is done with the modified Hidex equipment. In this point the difference between the measurement locations are studied and it is easier to move the cartridge to the location which are determined before. During these measurements, the washing protocol is also studied.

The repeatability is tested with high polymer concentration (c = 1000 µg/l), because it has highest and clearest signal. In this test five parallel PS cartridges are used. The chambers are washed with the buffer (V = 500 µl, room temperature (RT)). The flow rate is 500 ml/hr and flow is created with a syringe pump.

The measurement is done at four different places in the chamber (Fig. 3.8). The first measurement is done when the measurement spot is in the middle of the chamber. This point is controlled with the distance between the back edge of the adapter and the hole of the inlet of the cartridge (d = 15 mm). With help of this central point, every other measurement locations are determined. For the second measurement the measurement spot is moved 5 mm towards outlet, and for the third and fourth measurement, the measurement spot is moved from the middle point

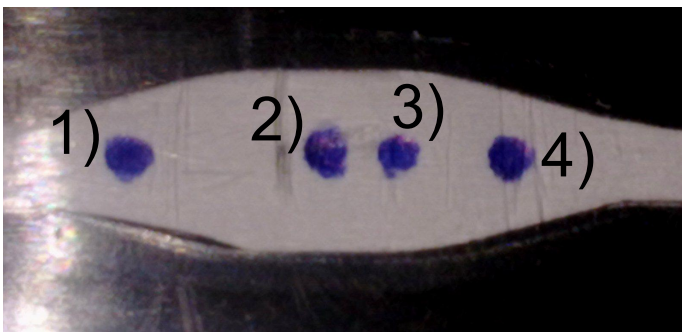


Figure 3.8. *The measurement locations from left to right: 1) 5 mm towards outlet, 2) middle of the chamber, 3) 2 mm towards inlet and 4) 5 mm towards inlet.*

2 mm and 5 mm towards inlet, respectively. With these different measurements locations are found out, that is the sample homogeneous and does the measurement place affect the results.

The background signal is measured in the middle of the chamber before the measurement is done. It is done with empty chamber and when the buffer (V = 50 µl) is in the chamber. The background is measured again after the sample measurement and washing to see how the wash affects the background signal. After first background measurement, the sample (V = 50 µl) is pipetted through the inlet in the measurement chamber and measurement is done in all four locations one after other.

After measurements, the chamber is washed with the buffer (V = 500 µl, T = RT, v = 500 ml/hr) and background is measured again with empty chamber and when buffer is in the chamber. This whole measurement is done with five cartridges to see are the measurement repeatable between the parallel cartridges.

After repeatability test, the volume of washing solution is increased from 500 µl to 1000 µl. When the volume is increased it affects the shear stress which Eq. 3.1 is shown below.

$$\tau = -\frac{6Q\mu}{h^2 w} \tag{3.1}$$

where Q is the flow rate, μ is the viscosity of the fluid, h is the height of the chamber and w is the wide of the chamber [47]. When the shear stress is high, particles are removed effectively. It is also known that flow rate is dependent on the volume which goes through the chamber in certain time. The improvement should affect the effectiveness of the wash.

The wash improvement is done so that the background is measured like before in the repeatability test. The sample is also same, but it is only measured in the middle of the chamber. Then the wash is done with new volume (V = 1000 µl), but other parameters are same.

3.2.3 Concentration measurements and detection limit

This measurement is done to see can different concentrations be measured with cartridge and what is the detection limit. This measurement is done with Victor^2, because after device familiarization and comparison is decided that device measurements are done with Victor^2 to get the exact values from static measurements.

The dilution series of the sample is done for the measurement. The conccentrations which are used, are 1000, 200, 40, 8 and 1.6 µg/ml. In this study also zero samples (buffer in the measurement chamber) are measured. From slope of the results of the zero samples can be determined the detection limit of the Victor^2

measurement.

There are three parallel cartridges for every concentration and zero samples. All three zero samples are measured first and then dilution series from the lowest concentration to the highest. The sample (V = 50 µl) is pipetted to the chamber and the cartridge is placed on its place and measured.

The measurement protocol of Victor^2 is determined so that measurement is done from the middle of the measurement chamber, which is decided to keep as measurement point. Victor^2 measures TRF counts five times from the same point, which is enough to count averages.

3.2.4 Re-usability of cartridge when wash between measurements

In this measurement is studied can the same cartridge be used several times, if the cartridge is washed between the measurements. This is done with two parallel cartridges and different concentrations of the dilution series (1000, 200, 40 and 80 µg/ml) are measured from the lowest concentration to the highest and again to the lowest one. The results of parallel cartridges are compared together to see that washing works correctly. Also this measurement is done with Victor^2 to get good and exact results.

During the test is seen does the dilution series act like the hysteresis curve when the series is measured from the low concentration to the large one and from the large to the low one. These back and forth rounds are performed four times. If the signal levels of next round are higher than during previous one, the previous round does affect the measurement results. Also if the hysteresis phenomenon is detected, then the previous larger concentration is affected the measurement results.

The sample (V = 50 µl) is pipetted in the chamber and the cartridge is measured with Victor^2. After the measurement is done the chamber is washed (V = 1000 µl, v = 500 ml/hr) with the help of syringe pump outside of Victor^2. The washing solution is sucked through the chamber. After the wash, the new sample is pipetted and measured. After four rounds, the whole test is redone with parallel cartridge.

3.2.5 Measurement with flow

The next step is to create the flow in the chamber during the measurement. It shows can the sample be taken directly from the water treatment process and direct the flowing sample through the chamber and measure it at same time. For that the tubing with the syringe pump and the valves are connected to the user interface, which controls the flow and the valves.

In this last measurement is studied can the measurements be performed using a

flowing sample. The measurement is performed so that corresponds the real sample measurement from the water treatment process. This measurement is done with the modified Hidex equipment, because the tubings for flow can be fixed through the equipment wall. Before the flow measurement, the concentration measurement and detection limit, and re-usability of cartridge measurement are done to get comparison values for the flow measurement. Those measurements are done same way as with Victor^2, but at least 10 measurement is measured from the measurement point (middle of the measurement chamber).

In Fig. 3.9 is shown the flow process which is created. The process and the washing solution chamber are connected to the main tube, which lead through the cartridge to the waste chamber. Before the main tube there is the other tube, which leads the flow directly to the waste chamber and the cartridge is bypassed. With the valves the path of the flow is steered during pumping and measurement. The pumping rate, when the fluid is moving in the tubing, is 500 ml/hr. Only exception is when the sample is in the chamber and it is measured, then the velocity rate is 1.25 ml/hr. With this low flow rate, most part of the excited particles are still on the measurement point when the emitted photons are counted by the modified Hidex equipment. When the samples is moved from the process chamber to the measurement chamber it does not matter what the flow rate is, but when the rate is high there is not so much exposure for UV-light from the lab room. The UV-light is also used to excite the electrons of the europium, so the emission ability can fade when the sample is exposured for UV-light from the lab.

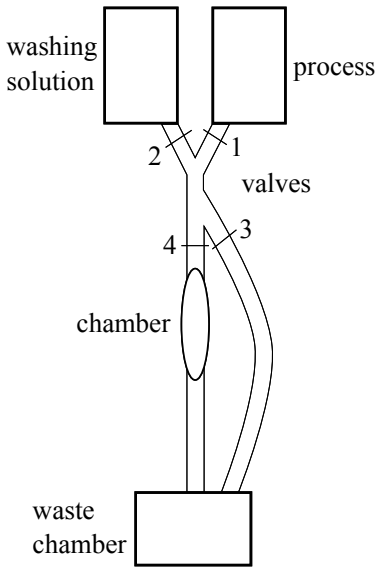


Figure 3.9. *The process chart. The valves will clamp the tubes when needed and the direction of the flow is changed.*

The valves and the pump are controlled with user interface. This user interface

(SyringeSequence) is created based on LabView software. In the software the user determines the sequences of the pumping operation. The states of the valves are also determined; are they open or closed. When needed sequences are determined to operate the syringe pump and the valves, the sequences are launched.

When the sequences are determined and right parameters are found, the measurement sequences can be saved and loaded again when needed. For example now the measurement sequence is saved its own file and the washing sequence its own. With complete measurement sequences the software is ready-to-use and easy to use. The modified Hidex equipment is controlled separately with its own software and it is launched at the same time as the user interface. The measurement setup can be seen in Fig. 3.10.

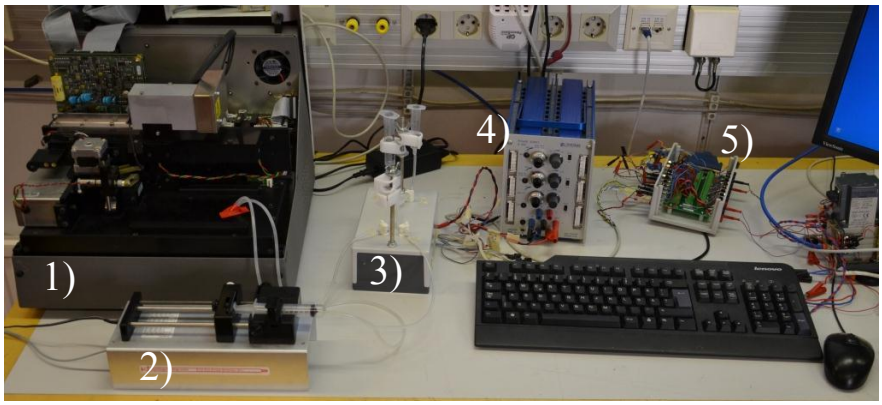


Figure 3.10. *The measurement setup. 1) the modified Hidex equipment, 2) syringe pump, 3) valves, 4) power supply and 5) signal port from computer to the valves. There is also computer for controlling the user interface and Hidex software.*

In the first step of the sequence, all the valves are closed and they clamp the tubes. The pump is reset. On the next step of the sequence the valves 1 and 4 are open (the valves 2 and 3 are closed) so the sample can flow (v = 500 ml/hr) from the process chamber to the measurement chamber to be measured. When the sample (V = 80 µl) is in the measurement chamber, the third step of the sequence starts with flow rate of 1.25 ml/hr. The same valves are open. In the fourth step of the sequence, the valves 2 and 4 are open (the valves 1 and 3 are closed) and the washing solution flows (v = 500 ml/hr, V = 1000 µl) through the chamber.

Before the actual measurement with the samples, the functionality of the tubes and the valves are tested with the dyed water. In these pre-tests is noticed that the rinse of the process chamber is good to do with the washing solution with generous volume of it.

In the flow measurement, the used concentrations are 1000, 200 and 40 µg/ml. The measurement order is 1000, 200, 1000, 40, 1000, 200, 1000, 40 µg/ml, and from every concentration is taken five samples one after the other. The five samples is

rationed with the pipette one by one ($V = 80$ µl) to the process chamber and there the samples flow to the measurement chamber and they are measured.

The same concentration is measured five times one after other, so the steps of the sequence from one to four are repeated five times. When the measurement of one concentration is done, the next sequence takes place, when the major washing is done. This is done always when the concentration is changed. The valves 1 and 3 are open (the valves 2 and 4 are closed) so the residues above the valve 1 can flow right to the waste chamber. The process chamber is rinsed ($V = 4000$ µl) and washing solution with residues flows (the valves 1 and 3 are open, the valves 2 and 4 are closed) directly to the waste chamber. After this wash, the next concentration can be measured.

3.2.6 Analysis methods

The both measurement equipment are programmed so that they measure several times at same point. On the results the values which are reported, are averages of these measurements (Eq. 3.2).

$$\overline{x} = \frac{1}{N} \sum_{i=1}^{N} x_i \tag{3.2}$$

where $\overline{x}$ is the mean (average) of the measured values, x_i are measured values and N is the number of the values. When the cartridges are compared together, the x_i stands for average of cartridge and N is the number of cartridges. The standard deviation is also calculated from these measurement values (Eq. 3.3).

$$SD = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - \overline{x})^2} \tag{3.3}$$

where SD is standard deviation, x_i are measured values, $\overline{x}$ is the mean value (average) of the measured values. For each concentration, which are measured during the re-usability of the cartridge test and the flow measurement, the standard deviation is determined with help of the equation above. From these analytical values can be determined the coefficient of variance (CV). In Equation 3.4 the formula gives directly the percentage value.

$$CV\% = 100\% \frac{SD}{\overline{x}} \tag{3.4}$$

where $CV\%$ is the coefficient of variance as percentage. For the biotechnological measurements, like measurement with polyacrylic acid, the coefficient of variance below of 10 % from tests is good.

From the results are also determined the limit of detection (LOD). The LOD-value determines in this study the lowest concentration which can be detected.

$$LOD = \frac{3SD_{dia}}{slope\ of\ x\text{-}values} \tag{3.5}$$

where SD_{dia} is the standard deviation of dialyse buffer measurement. In this study, the x-values are signal levels of each concentration and the curve is determined from these values. The slope of the curve is quotient between difference of x- and y-coordinates. The SD for dialyse buffer is calculated as in Eq. 3.3 from the zero sample values.

4. RESULTS

In this chapter the results are shown with the discussions. There is performed several experiments which give answers to the research questions which are: i) are the results repeatable between cartridges, ii) can different concentrations be measured and what is the detection limit, iii) can the same cartridge be used several times, if the cartridges is washed between measurements, and iv) can the measurements be performed using a flowing sample.

First there are results from comparison, which show Eu-STD solution measurement results and the difference between the equipment. Then there are the results of the repeatability test to see are the results repeatable between cartridges and between the measurement locations. Also the washing washing protocol is improved.

Next the signal counts of different concentrations and limit of detections are introduced. Then is studied, how the one cartridge behaves in static hysteresis test, when different samples are measured and only the washing is done between the measurements. In the last section is introduced the results when the measurement is done with the flow, such as in real water treatment process.

4.1 Device familiarization and comparison

The goal of the device familiarization and calibration is to get familiar with the equipment and the polymer. The measurement is done on two heights, and the signal level of sample is better when the cartridge is higher (from 4000-5000 counts to 7000-8000 counts). The comparison measurement is done with the known sample, when specific 1 nM sample should give the signal level as 1 000 000 counts. Table 4.1 shows the measured signal levels of Eu-STD solution. The samples are measured in the cartridge and the 96-plate with the modified Hidex equipment and Victor^2.

Table 4.1. *The signal levels of europium standard solution measured with the cartridge and the 96-plate by the modified Hidex equipment and by the Victor^2.*

	modified Hidex		Victor^2	
	cartridge	96-plate	cartridge	96-plate
TRF (counts)	1647	56644	122997	1528595

Measurement with the 96-plate is done to see, how the conventional measurement

system differs from the cartridge measurement. The signal levels of the modified Hidex equipment are risen when measurement is done with the 96-plate.

4.1.1 Discussion of device familiarization and comparison

It is known that the signal levels of the modified Hidex equipment are lower than signal levels of Victor^2. This basic difference is also between the original, unmodified equipment according the Hidex producer. In this study, the difference can be caused the cartridge placement in the modified Hidex equipment, because it is possible that the light spot does not hit to the measurement area optimal way. One reason for that is, that in this point the cartridge is adjusted in the equipment by measurer when Victor^2 does it automatically. Other reason for the difference can be the PMT. If Victor^2 has the red sensitized PMT, it will give better results.

As seen the measured signal levels with measurement equipment differs from each other quite much. One reason why the 96-plate signal levels are higher is that in the 96-plate the sample volume is 200 µl as said in the measurement instructions of Victor^2. In the case of the cartridge the fluorescent light has to go through the transparent plastic until it reaches the sample. Also the sample height is smaller in the cartridge, only 0,5 mm. But these reasons only explain the difference between the cartridge and the 96-plate.

Because of difference between the measurement results between the equipment, is decided to use Victor^2 to measure the signal levels and the LOD (limit of detection) -values of the samples. The modified Hidex equipment is used to measure repeatability between cartridges and measurement locations, and the sample with flow.

4.2 Repeatability between cartridges, measurement locations and washing protocol

In this measurement is studied, is the measurement repeatable between the cartridges and what kind of washing protocol is used. The measurement is done in five parallel PS cartridges. The polymer is measured with largest concentration of 1000 µg/ml in the four different locations; in the middle of the chamber, 5 mm towards the outlet and 2 mm and 5 mm towards the inlet from the middle of the chamber. This way is seen is there repeatablity between the different measurement locations. This is studied with the modified Hidex equipment.

In this repeatability measurement (Table 4.2), there are results of every cartridge and those values are averages of values which are measured in each measurement location. The average in the table is average of values of those five cartridges, and SD is the standard deviation from these five parallel averages. The coefficient of

variation (CV %) is calculated from these values. The results are shown in Table 4.2 and in Fig. 4.1.

Table 4.2. *Repeatablity between the cartridges and difference between measurement locations.*

		Cartridge							
		1	2	3	4	5	average	SD	CV%
TRF	In the middle of the chamber	8334	8511	8426	7663	10469	8680	1054	12.1
	5 mm to outlet	8028	7415	7857	7677	8912	7977	567	7.1
	2 mm to inlet	8966	8308	8293	7177	9106	8370	763	9.1
	5 mm to inlet	8537	8113	8098	7217	6150	7623	953	12.5
	background	115	116	128	130	118	121	6.8	5.6

There are differences between the cartridges and the variation coefficient between the cartridges varies from 7 to 13 percentages. There is also little bit variation between the measurement locations as can be seen on Fig. 4.1.

The measurement locations 5 mm to outlet and 5 mm to inlet are geometrically similar with each other. The signal levels act quite differently between these locations despite of the geometrical similarity. Values of the cartridge 5 differs from other cartridges quite much despite the measurement location.

The background signal levels of all cartridges are nice, also the average background signal level is nice (table 4.2). The variation is good, less than 6 %. The washing improvement is done after the repeatability measurement to see does the

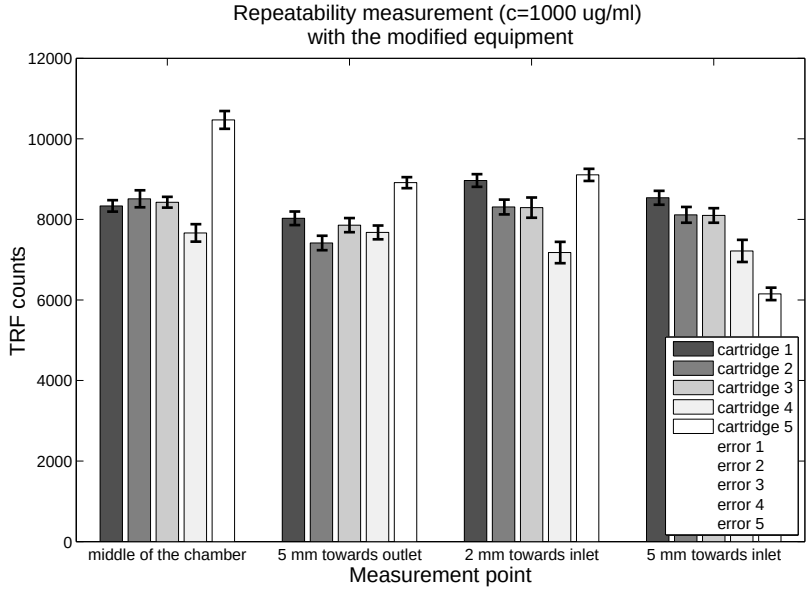


Figure 4.1. *Repeatability between the cartridges and difference between the measurement locations.*

larger washing volume affect the background signal.

In the washing improvement, the used sample concentration is 1000 µg/ml. The washing is done with the volume of 1000 µl, so the volume is doubled and the flow rate is kept as 500 ml/hr and the solution is at room temperature. From table 4.3 can be seen how the background signal is acted during the improvement measurement.

Table 4.3. *Washing improvement with the modified Hidex equipment.*

		Cartridge							
		1	2	3	4	5	average	SD	CV %
TRF	background before	108	82	117	101	109	103	13	12.6
	1000 µg/ml	10113	9836	9686	10410	13521	10713	1594	14.9
	background after	120	123	87	86	104	104	18	16.9

Overall with every cartridge can be said that the signal level of the background signal is decreased back to its initial level after the sample measurement and the washing. The cartridges 1 and 2 are exceptions and the signal levels seem to increase little bit, but those increases are in the limits of the variation.

4.2.1 Discussion of the results of the repeatability between cartridges and washing protocol

The repeatability between cartridges seems to be good. There is only one cartridge which results stood out from others. But overall the results of parallel cartridges can be compared together. Of course there is same variation, but it can also be caused by the fabrication process of cardridges. There can be some differences on the plastic after injection molding which affect the optical properties of the transparent plastic.

It seems that for the polymer, there is not any measurement location which is clearly the best one. In this point is chosen, that the middle of the measurement chamber is used as measurement location. It gives also little bit afford to miss exactly the midpoint of horizontal axis without that the measurement point does hit outside the chamber or channel area and measurement is failed. One possibility is also to form geometry which is wide-channel-like. That also gives possibility to the measurer miss the midpoint little bit. This study is continued with the measurement chamber with inlet and outlet channels.

In the washing tests only one improvement is done in this phase and it is the increase of the volume of the washing solution which is doubled. Because the velocity is kept the same, the washing takes twice as much time as before. There is little bit improvement and the new washing volume is used from now on. The shear stress is increased based on Eq. 3.1, and more particles are removed from the surface. The shear stress is dependent on the flow rate, and the flow rate is dependent on the volume which goes through the chamber in certain time. Based on this, the

increase of the flow rate is not necessary at this point because it does not bring any remarkable benefit. The washing protocol which is used during the measurement now on: the washing solution (buffer) is at room temperature, velocity is 500 µl/min and the washing volume is 1000 µl.

4.3 Concentration measurements and detection limit

This measurement is performed to study can different concentrations be measured and what is the detection limit. This is done with Victor^2 to get exact values from static measurement. The dilution series is done from the sample and measured concentrations are from the series. Also zero samples are measured. There are three parallel cartridges for every concentration. The sample is pipetted through the inlet in the chamber and the signal levels are measured.

The measurement is done four times with the same cartridges and the cartridges are washed between the measurement times. In Table 4.4 can be seen results from these measurements and in the lowest part of the table are shown the average results from these four measurements.

Table 4.4. *The signal level measurement with the Victor^2.*

			Concentration µg/ml						LOD
			0	1.6	8	40	200	1000	(µg/ml)
TRF	1	Av.	714	2676	10534	48843	241005	1232238	0.20
		SD	82	19	274	449	2190	14057	
		CV %	11.5	0.7	2.6	0.9	0.9	1.1	
	2	Av.	625	2917	12157	59473	289995	1447845	0.04
		SD	21	63	130	154	4922	3374	
		CV %	3.3	2.2	1.1	0.2	1.7	0.2	
	3	Av.	727	2763	11335	52122	255983	1250181	0.18
		SD	74	60	494	489	1753	6658	
		CV %	10.1	2.2	4.4	0.9	0.7	0.5	
	4	Av.	585	2720	10807	50543	240857	1200009	0.09
		SD	38	72	336	1148	796	16474	
		CV %	6.4	2.6	3.1	2.3	0.3	1.4	
	All four	Av.	663	2769	11208	52745	256960	1282568	0.13
		SD	29	23	151	419	1770	6147	
		CV %	4.4	0.8	1.3	0.8	0.7	0.5	

In Table 4.4 the averages (Av.) of each concentration are averages of three parallel cartridges. The standard deviation (SD) is also between these three parallel cartridges. In the last part of the table in the 'all five' columns, the average is average of the averages of four values above and the standard deviations are from the four average values. The limit of detection (LOD) values is the lowest concentration which can be detected by each cartridge. And in the last row the LOD value is average from four previous values. In Fig. 4.2 are shown the signal levels and variations of both measurements per concentrations.

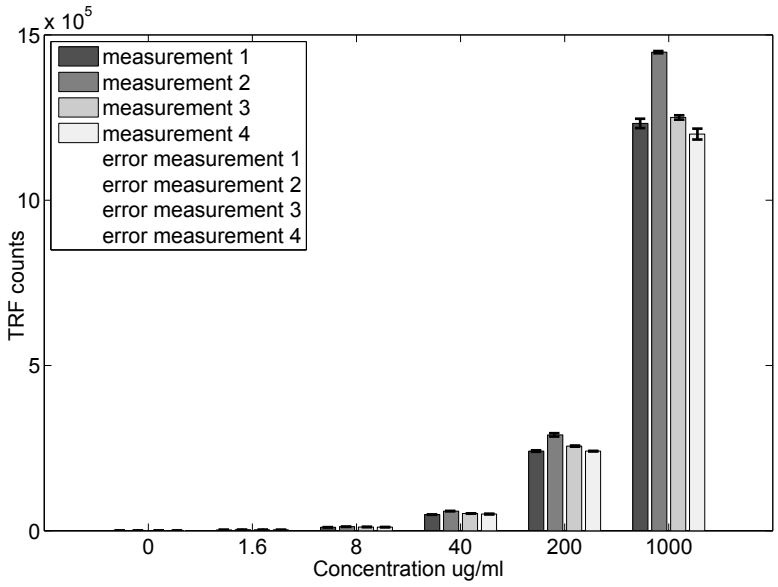


Figure 4.2. *Concentration measurement with Victor*^{2}.

The signal level measurement by the Victor^2 give signal levels which are increased proportionallu to concentration increase. The variation is small on every concentration, only the zero samples are exception but it is still very good. The LOD value is very good on this static measurement by the Victor^2.

4.3.1 Discussion of the results of concentration measurements and detection limit

The values of concentration measurement with Victor^2 are very good and when the results from the next test look same, can be seen that the same cartridge can be reused when the washing is done between the measurements. The values of the CV % on the lowest row show that the variation between measurement times is small, so it also supports that the cartridges can be reused. Also this tells that the different measurement times can be compared. The CV % -values of section 4.2 are maximally 20 times larger. The measurement of section 4.2 is done with modified Hidex equipment because the difference between the measurement locations are also studied and it is easier to move the cartridge to the locations which are determined in section 3.2.2.

The LOD -values are very good in all measurement times. Concentrations, which are measured from the processes, are varied between different processes. In the water treatment process the detection level is ppm (mg/l = µg/ml). So the determined LOD -value between the cartridges (0.13 µg/ml) is also very good. Toxity of polymaleic acid derivative (copolymer of maleic and acrylic acids) for fish is 200 - 17 000

mg/l (= 200 - 17 000 µg/ml) and for water flea it is over 200 mg/l (= 200 µg/ml) [35]. It seems that this kind of detection system, where the microfluidic cartridge is used with the TRF, can be used in water treatment process.

On this far can be said that the cartridge works as measurement platform and different cartridges can be compared together. In the next measurement, the presumption is that the values from different concentrations, the variation and the LOD -values stay good. Also the affect of the previous sample to the next one is tested.

4.4 Re-usability of cartridge when wash between the measurements

In this measurement is used one cartridge. During the measurement is studied can the same cartridge be used several times, if the cartridge is washed between the measurements. This is performed with Victor^2. In this test the dilution series is measured from the lowest concentration to the largest one and back from the largest to the lowest. This circle is done four times for every cartridge and with two parallel cartridges. With this test is seen does the previous measurement round affect the next one and does the previous larger concentration affect the measurement results despite of the washing.

The re-usability test is done with the Victor^2 and the results are good. The signal levels are high as they should be and the variation is small. The results are shown in Table 4.5 below.

Table 4.5. *The re-usability of cartridge, when wash between measurements, done with Victor^2.*

			Concentration µg/ml						
			8	40	200	1000	200	40	8
TRF	c3	Av.	10961	48191	213619	1114319	235102	48917	10815
		SD	550	415	27423	22335	11193	1242	646
		CV %	5.0	0.86	12.8	2.0	4.8	2.5	6.0
	c4	Av.	12387	56094	269142	1362184	271815	55851	12357
		SD	299	1535	3861	36884	9101	1139	324
		CV %	2.4	2.7	1.4	2.7	3.3	2.0	2.6

From Table 4.5 can be seen that the values stay good. The variation of these Victor^2 results is small. When the dilution series is measured with cartridge 4 from the lowest to the highest concentration the variation is less than 3 %, and when measured from up to down the variation is 3.3 % at highest. With cartridge 3 the variation per concentrations is quite same despite few exceptions.

As can be seen from the Appendix A on Fig. A.1, there are not almost any hysteresis in the measurement results of the cartridges. On Fig. A.1 can be also

Table 4.6. *The re-usability of cartridge, when wash between measurements, done with Victor*^{2}.

			Concentration µg/ml			
			8	40	200	1000
TRF	c3	Av.	10888	48554	224361	1114319
		SD	561	941	22536	22335
		CV %	5.2	1.9	10.0	2.0
	c4	Av.	12372	55973	270479	1362184
		SD	289	1258	6628	36884
		CV %	2.3	2.2	2.5	2.7

seen that there are just small differences between the rounds. These differences are all in the variation.

When the all same samples are taken care in both cartridges, four concentration values per one cartridge are derived. This way is got the average and the variation for one concentration per cartridge. These results are shown in Table 4.6.

In the case of the cartridge 3 the variation per concentration is quite high in the case of $c = 200$ µg/ml, but it is not crucial. Otherwise the variations of other concentrations are good in the cartridge 3 and 4. This can be seen from Fig. 4.3.

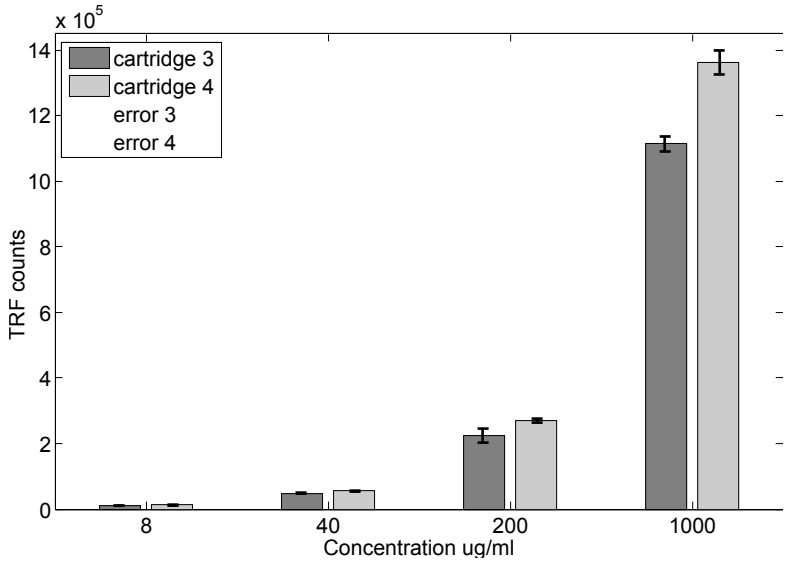


Figure 4.3. *In the picture is signal levels of the re-usability of cartridge measurement which is measured with Victor*^{2}*. The values are per each concentration. The wash is done between the measurements.*

As can be seen from the bar diagram, between the cartridges there are difference, which stands out markedly when measured with largest concentration.

4.4.1 Discussion of the results of re-usability of cartridge when wash between measurements

The main point at this test is to find out how the previous sample affects the next sample during the measurement when wash is done between the different samples. The hysteresis test results from the Victor^2 are excellent. The variation is small and the signal levels are high. There are no remarkable differences between measurement rounds, so the measurements can be performed again with the same cartridge. Also the hysteresis phenomenon is hardly seen, so even the previous sample do not affect the next sample, because of the successful washing.

In this point it seems that the cartridge can be used several times, when it is washed between the measurements. Even the previous sample does not affect the next measurement result. There are some differences between the cartridges, but it is not crucial. One possible explanation is, that is caused by the molding and laser welding problems which are occurred in these cartridges. Also the location of the cartridge can effect, because there is also little possibility for variation when the cartridge is placed on the adapter of the Victor^2. If the cartridge is little bit different location and there is exception caused of molding on the plastic, it can affect the signal level. These can explain the difference between the cartridges, because there is the little difference also in the case of lowest concentration and the difference increase when the concentration is increased.

As can be seen from the round curves in Appendix A on Fig. A.1, that the washing is working, and the previous sample does not affect the next sample. This can be said from figure, because if there is any affect it is shown as hysteresis phenomenon. Now the curves of rounds are on the same track all time.

Based on these results can be said, that the cartridge works, and same cartridge can be used several times when the measurement chamber is washed between the measurements. Next can be studied how the measurement works when sample is flowing during the measurement.

4.5 Measurement with flow

The last step is to study can the measurements be performed using a flowing sample. This is done with the modified Hidex equipment. The sample is measured same way how it is measured in the real sample analysis in water treatment system. Before the actual flow measurement, the signal values for modified Hidex equipment is studied. It is done with the same kind of measurements which are done with Victor^2 during the study. These static measurement results can be compared to the results from flow measurement to see does the measurement with flow work.

4.5.1 Concentration measurement and detection limit

First the results from the concentration measurements with the modified Hidex equipment. Also the LOD is determined. The results are seen in Table 4.7 and in Fig 4.4. The measurement is done two times with same cartridges which are used when the measurement is done with Victor^2.

Table 4.7. *Two concentration measurement with the modified Hidex equipment. On the bottom of table are the average, the SD and the CV % of both measurement times. The LOD values on the most right column.*

			Concentration µg/ml			LOD
			8	200	1000	(µg/ml)
TRF	1 measurement	Av.	56	1507	7789	3.3
		SD	31	127	467	
		CV %	56.1	8.4	6.0	
	2 measurement	Av.	79	1576	8186	3.0
		SD	31	218	1091	
		CV %	38.9	13.8	13.3	
	Both two	Av.	67	1542	7987	2.1
		SD	16	48	281	
		CV %	23.5	3.1	3.5	

In the case of first and second measurements, on the average rows the values of each concentration are averages of three parallel cartridges. The standard deviation (SD) is taken from these three parallel average values. On the last row the values of both are so that the average is average of these two measurement times and the SD from the averages.

The limit of detection (LOD) -values are quite high when compared to the LOD -values of the static measurement of the Victor^2. But this is predictable, that the LOD -values are high because of the low signal levels and larger variation of the values when compared to the Victor^2. Despite the LOD -values are worse than with Victor^2, they have same magnitude (/ml) as concentrations in water treatment process

As can be seen from Fig. 4.4 the signal levels in both measurements act same way. The signal level of the concentration of 8 µg/ml looks so low, that it hardly stands out from the background.

In the case of the modified Hidex equipment, the variation of the lowest concentration is in both cases high. The variations of the two largest concentrations are below 10 % in the first case and below 14 % in the second case. Commonly in the biomeasurements the variation below 10 % is good, so can be said that with the largest concentration the results are good, and with $c = 200$ µg/ml the variation should be little bit better.

The variation between two measurement times on two largest concentrations is

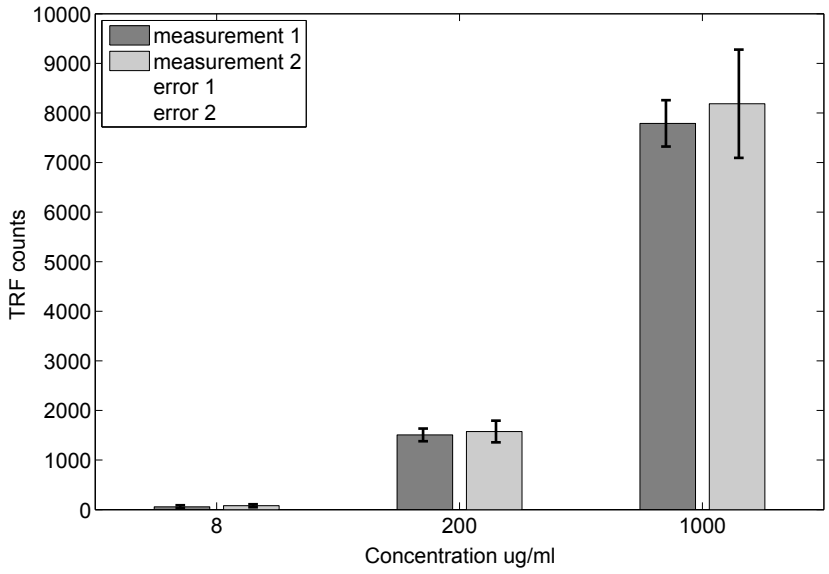


Figure 4.4. *Concentration measurement with the modified Hidex equipment.*

small. This is good to know that the measurement is repeatable although the same cartridge is used and washed between the measurements. The variation between three different cartridges is larger than the repeatability of the one cartridge (Table 4.7).

4.5.2 Re-usability of cartridge when wash between measurements

The results of re-usability of cartridge measurement are shown in Table 4.8 and in Fig 4.5. Now the signal levels are compared so that in the case of every cartridge the same concentrations are combined. As can be seen the signal levels of each concentration vary when compared between the cartridges. In most cases the variation is lowest when the largest concentration is measured so the signal level is high. In the limits of the variation the signal levels are not affected by the previous sample. So the washing is good enough to reuse the cartridge.

The results are divided to five parts, one for each cartridge. For each concentration the average is determined from all values which are measured during four rounds despite is the hysteresis circle measured from down to top or from the top to down. And naturally the standard deviation is determined from these values.

As can be noticed the signal levels increase nicely when concentration is increased. In the case of most cartridges the variation is smallest when the signal level is highest. When the cartridges are compared, is noticed variation between them. In Fig. 4.5 can be seen the difference between cartridges. The large CV % -values are caused

Table 4.8. *The re-usability test with the wash, measured with the modified Hidex equipment. The values are summary of each concentration in each cartridge.*

			Concentration µg/ml					
			0.32	1.6	8	40	200	1000
TRF	c1	Av.	76	77	101	237	1008	5811
		SD	17	29	57	129	465	1525
		CV %	22.2	37.0	56.5	54.4	46.1	26.2
	c2	Av.	65	79	115	281	1288	6249
		SD	17	23	35	96	429	1925
		CV %	26.6	28.8	30.5	34.3	33.3	30.8
	c3	Av.	117	126	182	482	1929	9806
		SD	23	27	40	88	334	1602
		CV %	19.5	21.4	22.1	18.1	17.3	16.3
	c4	Av.	50	60	101	275	1103	6577
		SD	13	11	30	73	486	1089
		CV %	26.9	18.9	30.0	26.5	44.0	16.6
	c5	Av.	29	38	78	319	1124	4970
		SD	5	8	19	231	222	1453
		CV %	17.6	21.4	24.7	72.6	19.8	29.2

by the modified Hidex equipment itself. With Victor^2 the variation is about 5 % or below, but now the variation is over ten times larger in the worst cases.

In the Appendix B in Table B.1 are shown the signal levels from the measurement in the order which those are measured. On the top of the table are concentrations which are used in the dilution series. The values on the left side of the concentration of 1000 µg/ml are values when circle is measured from the lowest concentration to the largest one, and on the right side when is measured from the largest to the lowest

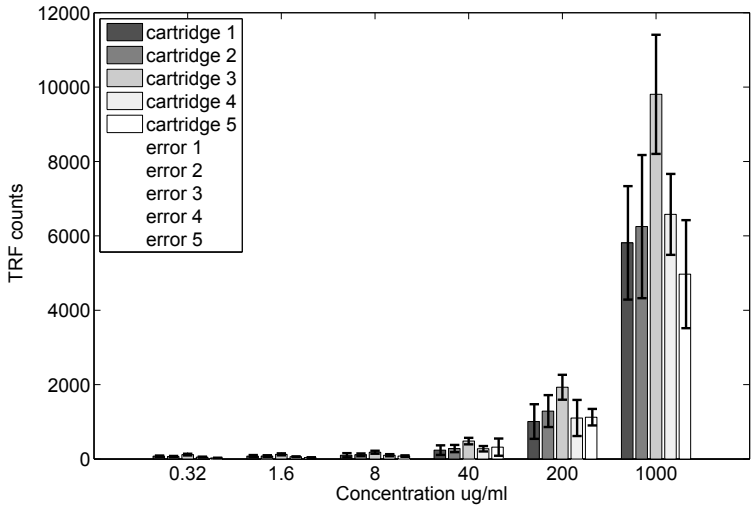


Figure 4.5. *Re-usability measurement with the modified Hidex equipment. The measurement is done with five parallel cartridges.*

concentration. The table is divided into five parts, one part for each cartridge. The average of every concentration is average of the four measurement circles, the standard deviation is from these four values and the CV% is determined from these previous values. These more specific values act the same way as the values of Table 4.8.

As can be seen there are quite large difference between the cartridge. The differenca can be caused that the cartridge is placed manaually on the adapter. The cartridge 3 stoods out in every concentration, so it is possible that it has been displaced somehow.

In the case of the modified Hidex equipment, the actual signal levels in the re-usability measurement are increased how they supposed to. In the limits of the variation, the previous sample does not affect the next sample (Table B.1), so the washing is working correctly. The variation is quite large, but most of it is caused the quite low signal levels and the modified Hidex equipment itself.

From the re-usability test results can be seen that with the both measurement equipment the signal levels act same way despite the fact that the signal levels of the modified Hidex equipment are low. The results from the modified Hidex equipment are good to know before the flow measurement.

4.5.3 Measurement with flow

The last step of the research is to create measurement with flow and measure the sample like it is done in the real life. During the measurement, the five sample series per concentration is measured, measurement chamber is washed, and then the next concentration of five samples is meaasured. During the measurements and signed when the values are analyzed, is noticed that the first value from five value series is markedly lower than others. This is recurred in every measurement, so the averages shown in Table 4.9 and Table C.1 are calculated without first value (only four values per series). This can be done, because the measurement is done in the waste water process and the sample can be taken several times from the process.

As can be seen from Table 4.9, on the first measurement time zero samples are not measured and instead of $c = 40$ µg/ml, $c = 8$ µg/ml is measured. It is noticed that signal levels of the 8 µg/ml are quite close of the signal level of zero sample and background level. That is reason why the $c = 40$ µg/ml is selected to the lowest concentration which stood out clearly from background and zero sample values.

In both measurements the average values are average of series of four samples and standard deviation and variation coefficient are determined from those values. In Table 4.9 below the results per concentrations are observed, and the first values of the series are left out. In the last row, the two measured times are compared together.

Table 4.9. *The flow measurment test with the modified Hidex equipment. The values are averages, standard deviation and variation of every concentration.*

			Concentration µg/ml			
			0	1000	200	40/8
TRF	1	Av.	-	3606	670	-/90
		SD	-	221	117	-/8
		CV %	-	6.1	17.4	-/8.9
	2	Av.	66	3762	739	199/-
		SD	9	568	122	23/-
		CV %	13.8	15.1	16.1	11.8/-
	both two	Av.	66	3723	704	199/90
		SD	9	388	121	23/8
		CV %	13.8	10.4	17.1	11.8/8.9

As can be seen in the first measurement time the signal levels of the concentration of 1000 µg/ml are from 3400 to 3800. On the second time the values are from 3400 to 4400. The highest value is only because of the first measurement series. In the case of the lower concentrations the signal levels in both measurement times are more constant. In Fig. 4.6 is shown the signal levels and variations of the concentrations of both measurement times.

As can be seen from Fig. 4.6 the signal levels of each concentration are quite same in the both measurements. The variation of the concentration of 1000 µg/ml on the second measurement time is caused of the first measurement series of the $c =$ 1000 µg/ml. During this second measurement is noticed, that the signal levels of c

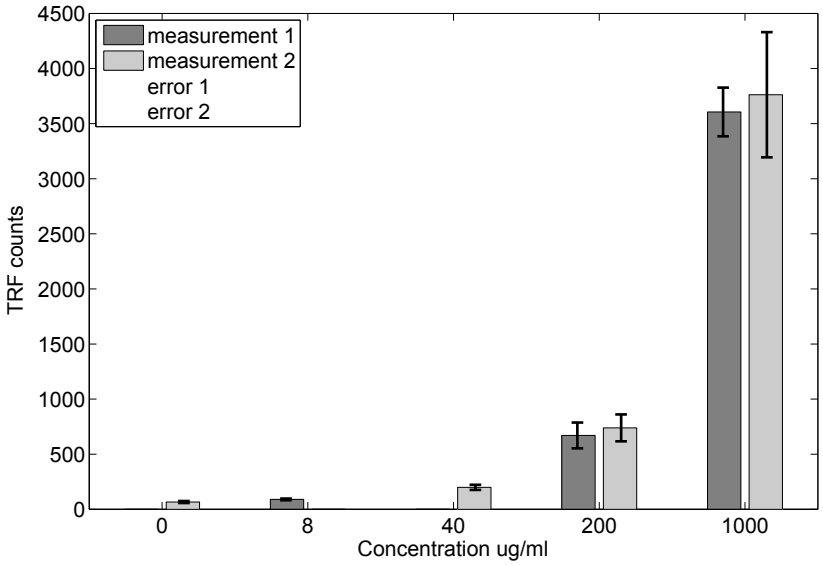


Figure 4.6. *The signal levels of the flow measurement, which is done with the modified Hidex equipment. The measurement is done twice with the same cartridge.*

= 8 µg/ml and zero sample are basically the same, so with this system the first clear measurement result with the flow, is signal level which is caused the concentration of 40 µg/ml.

The measurement results as detail are seen in the Appendix C in Table C.1 in that order where those are measured. Also on those results the first value of five value series is left out.

4.5.4 Discussion of the measurement with flow

The signal levels of every measured concentration are as high as are expected. On the second measurement time the signal level of first five sample series of 1000 µg/ml are higher than signal level of the other parallel series. After the first series the signal level of 1000 µg/ml is decreased back to the expected level.

At the first measurement time the variations of measurement series are small. On the second time the variations are little bit higher but still good. It is also noticed that the variation is smaller when the measurement is done with flow. It can be because the polymer particles do not adsorb to the chamber material so effectively.

From the measurement times can be seen that the measurement can be repeated with the same cartridge. The signal levels of each concentration are similar in both measurements. The variation between these measurements is little bit over 10 %, when the variation coefficient below 10 % is good in biotechnical measurements. When the measurement equipment is better optimized for the cartridge flow measurement, the signal levels increase and the variations should be lower.

Overall the flow measurement worked fine and the measurement results act like expected. This shows that the TRF measurement can be performed during the flow. It is good to get more than one sample from certain process, because after the wash of process simulation chamber, the signal levels seem to be down. But it is not a problem in the waste water process.

5. CONCLUSIONS

The target during the NucleoTracker-project is to study the measurement system, how the polymer tracking measurement is performed with flowing sample. First the device familiarization and comparison is done. Then the repeatability between cartridges and measurement locations and washing protocol is studied. Next concentration measurement and detection limit, and re-usability of cartridge when wash between measurements are done with Victor^2. The last measurement with flowing sample is done with the modified Hidex equipment.

During device familiarization and comparison, the modified Hidex equipment is got familiar. The right height for the measurement chamber is found (11 mm). It is noticed that when the cartridge is higher, the excitation light spot is sharper and it can more effectively excite the electrons of the europium. Unfortunately there is not any space to get the cartridge higher because of the connections and tubings. The comparison is done with cartridge and with 96-well plate, which is normally used in fluorometric measurements. The comparison measurement is also performed with Victor^2. The result of the comparison is that the measurements which handle values of sample and fuctionality of measurement system (cartridge and equipment), are done with Victor^2. The modified Hidex equipment is used in the repeatability test between cartridges and measurement locations, and in the flow measurement.

Repeatability between cartridges test is done with five parallel cartridges and on four different locations on measurement chamber area. During this test is noticed that results from parallel cartridges can be compared together. It is also studied that the location of measurement point on the measurement area do not affect the results. So the measurement area can be also wide channel. The most important thing is that the excitation light spot is on the measurement area and there is not risk that it hits on the plastic outside of the area. So in the future the cartridge can be even simpler with only one wide channel where the measurement is done.

In concentration measurements samples are measured with Victor^2. Different concentrations from dilution series are measured. For each concentration there are three parallel cartridges. Also zero samples with buffer is measured for definition of detection limit. LOD-values are good and cartridge works as measurement platform (Table 5.1). In the water treatment process the needed detection level is ppms (mg/l = µg/ml), so can be said that LOD -values of the static Victor^2 measurement is very

Table 5.1. *The conclusion of CV % -values of static measurements with Victor^2 and the modified Hidex equipment.*

			Concentration µg/ml						LOD
			0	1.6	8	40	200	1000	(µg/ml)
CV %	1	Victor^2	11.5	0.7	2.6	0.9	0.9	1.1	0.20
		Hidex	-	-	56.1	-	8.4	6.0	3.3
	2	Victor^2	3.3	2.2	1.1	0.2	1.7	0.2	0.04
		Hidex	-	-	38.9	-	13.8	13.3	3.3
	3	Victor^2	10.1	2.2	4.4	0.9	0.7	0.5	0.18
		Hidex	-	-	-	-	-	-	-
	4	Victor^2	6.4	2.6	3.1	2.3	0.3	1.4	0.09
		Hidex	-	-	-	-	-	-	-
	Average	Victor^2	4.4	0.8	1.3	0.8	0.7	0.5	0.13
		Hidex	-	-	23.5	-	3.1	3.5	2.1

good and the values from the modified Hidex measurement have same magnitude, despite the LOD -values are over ten times greater. The polymer with Eu-label can be measured reliably from the cartridge. This kind of detection limit promises good for future development. The same trend can be seen the CV % -values of individual measurement times and also between the different measurements.

During re-usability of cartridge when wash between measurements is studied, can the same cartridge be used several times if the measurement chamber is washed (V = 1000 ul, v = 500 ml/hr, T=RT) between the measurements. This is done with Victor^2. This kind of measurement system works and it confirmed the need of washing between the measurements, because it is noticed, that the Eu-labeled polymer does adsorb to the cartridge material, already during the washing protocol improvement. When the washing is done between the measurements, the values corresponds the values from the concentration measurements.

The last phase is to study measurement with flow and is it possible. Now the modified Hidex equipment is used. The cartridge is connected with tubes to the process and wash chamber and to the waste chamber. The waste chamber is cylinder of syringe, and it is fastened to the syringe pump. The syringe pump is connected to the outlet of the cartridge. In Table 5.2 can be seen that the variation is still larger than when the measurement is done without flow with Victor^2. This difference is caused the modified Hidex equipment itself.

From each concentration is done five sample series measurement, and after the series the concentration is changed. Between the series the residues of previous concentration are rinsed to the waste chamber. It is noticed that the first sample of the series after the concentration change do not give right values, but the next one gives. That is why the first measurement is left out from the calculations and only four samples are taken care. When this kind of environmental POC measurement is done with the waste water it does not matter how many samples is taken from

Table 5.2. *The CV % -values from the flow measurement with the modified Hidex equipment.*

		Concentration µg/ml			
		0	1000	200	40/8
CV %	1	-	6.1	17.4	-/8.9
	2	13.8	15.1	16.1	11.8/-
	both two	13.8	10.4	17.1	11.8/8.9

the one process. If the POC measurement is done for example with the patient, the first measurement should be as good as others and the measurement can be done with one sample.

Flow from the process and wash chambers is controlled with valves. The valves and syringe pump is controlled with user interface and the modified Hidex equipment is controlled its own software. The interface and Hidex software are quite easy to use. In the future, the properties of both interfaces should be combined. All steps of flow control and TRF measurement should happen with one start button.

Overall it seems that the cartridge can be used to measure the polymer amount in the waste water process. The needed detection limit is µg/ml, and the average detection limit in static measurement is 0.13 µg/ml. To get even better results there are some things what can be improved. The measurement area of the cartridge can be improved so that the height of the sample in the cartridge is larger. Then there is more Eu-labeled polymers in the measurement area and the signal levels are higher because more excited labels. In same point the PMMA cartridge is tried to use instead of the PS cartridge, but the laser welding did not turn out well, so the cartridge material is kept as polystyrene during the tests.

Now the tubing is connected to the cartridge inlet and outlet on the top of the cartridge, and the tubes take about 1 cm space above the cartridge. When the cartridge is higher, the measurement spot is smaller and sharper and the excitation is more effective. If there is possibility to modify the cartridge so, that the inlet and outlet holes are on the sides of the cartridge, then the connectors and the tubings do not need space and the cartridge can be placed higher.

The connectors between the tubes and inlet and outlet holes are little bit problematic. They are done with help of connectors and tips of pipette which are sealed with the silicone tube. The connector system can be improved to be more tightly and better sealed, so the vacuum can be created better. One possibility is modify the cartridge so, that the tubings can be directly connect to the cartridge without separate connectors.

The equipment itself should have the possibility for the tubing so, that the extra light from the outside does not affect the measurements. The UV-light from room where the measurement is done, excitates electrons of europium and it looses its

effectiveness. The tubing which is used during the tests is silicone tubes which are known their absorption ability. Also UV-light goes through the tube. The teflon tubes are tried to inhibit the absorption, but it do not affect the measurement results. So when the tubes are not too long, the silicone tubes are fine, despite of UV-light and absorption.

The measurement equipment and the place of the cartridge can be also improved. The equipment itself should have capability to measure the cartridge (excitation light go through plastic, sample and then it reflects back) and the place of the cartridge should be properly optimized. If the cartridge is modified and the place of the cartridge in the measurement equipment is optimized, the cartridge is on its place for sure every measurement time. This should decrease the difference between measurement times. Also the physical size of the equipment should be smaller if the measurement is wanted to be done near of the actual water treatment process.

During this project is successfully studied that microfluidic cartridge can be used to measure polymer residues from water process samples. The values from static measurements are clear, CV % -values are small and the detection limit is below the concentrations of real process. It also studied that the TRF measurement can be done during the flow. There is still way to the ready and working system, when all parts are optimized to the one system. But if this concept is developed more in the future, there can be huge market for example in Africa. The water treatment process is not as complicated than process introduced in this sudy, but more robust and simple. With this kind of measurement system, the water quality can be measured easily and quickly to see is the water pure enough to use or does it cause health issues.

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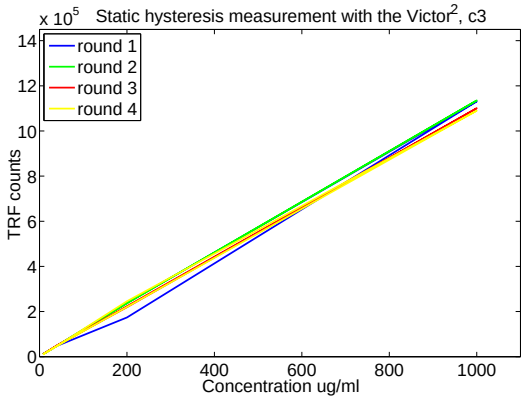
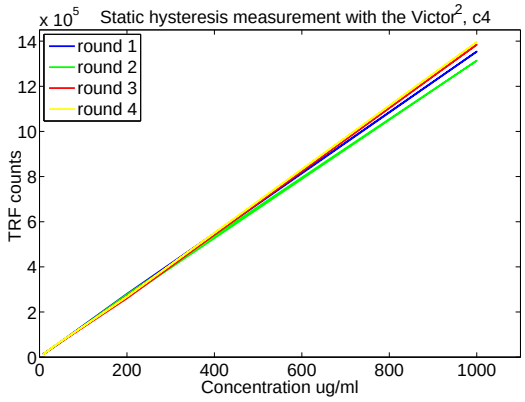
A. APPENDIX A: THE HYSTERESIS FIGURE OF THE VICTOR^2



(a)



(b)

Figure A.1. *In the picture are two cartridges and four measurement rounds for each cartridge. The re-usability of cartridge test is performed with Victor^2. In both cases there is hardly any affect of hysteresis phenomenon.*

B. APPENDIX B: DETAILED RESULTS FROM THE RE-USABILITY OF THE CARTRIDGE WITH THE MODIFIED HIDEX EQUIPMENT

Table B.1. *The re-usability of the cartridge with wash between measurement performed by the modified Hidex equipment.*

			Concentration µg/ml										
			0.32	1.6	8	40	200	1000	200	40	8	1.6	0.32
TRF	c1	Av.	73	73	116	260	1050	5811	966	215	85	82	80
		SD	19	34	68	160	549	1525	446	109	48	26	16
		CV %	26.7	47.2	59.0	61.8	52.3	26.2	46.1	50.6	55.8	31.7	20.2
	c2	Av.	65	79	122	291	1342	6249	1235	272	108	79	66
		SD	18	27	38	91	462	1925	457	115	35	22	19
		CV %	27.8	33.7	31.5	31.4	34.4	30.8	37.0	42.2	32.8	28.2	29.5
	c3	Av.	115	126	182	472	1865	9806	1993	493	181	126	120
		SD	29	32	50	108	426	1602	261	77	35	25	20
		CV %	25.0	25.8	27.6	22.9	22.8	16.3	13.1	15.6	19.5	20.2	16.3
	c4	Av.	44	56	94	289	1028	6577	1177	260	108	64	55
		SD	17	10	32	63	453	1089	574	89	31	12	8
		CV %	37.5	17.7	34.2	21.7	44.1	16.6	48.8	34.1	28.9	19.3	13.8
	c5	Av.	27	42	91	253	1211	4970	1037	384	66	34	31
		SD	6	7	15	74	155	1453	267	329	15	8	3
		CV %	23.4	17.1	16.3	29.1	12.8	29.2	25.7	85.7	22.9	22.7	10.6

C. APPENDIX C: MEASUREMENT WITH FLOW MEASUREMENT RESULTS IN THE ORDER WHERE THEY ARE MEASURED

Table C.1. *The measurement with flow measurement with the modified Hidex equipment. The values are from four sample series in the order where they are measured.*

			Concentration µg/ml								
			0	1000	200	1000	40/8	1000	200	1000	40/8
TRF	1	Av.	-	3687	756	3759	-/90	3566	584	3412	-/90
		SD	-	109	12	209	-/7	35	110	307	-/10
		CV %	-	3.0	1.6	5.6	-/7.9	1.0	18.8	9.0	-/11.1
	2	Av.	66	4412	698	4033	202/-	3504	780	3413	197/-
		SD	9	338	133	161	27/-	364	112	132	23/-
		CV %	13.8	7.7	19.1	4.0	13.3/-	10.4	14.4	3.9	11.8/-
	both two	Av.	66	4050	727	3896	202/90	3535	682	3412	197/90
		SD	9	1967	1644	2110	27/7	242	146	219	23/10
		CV %	13.8	48.6	226.2	54.2	13.3/7.9	6.8	21.5	6.4	11.8/11.1